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ELECTROMYOGRAM STUDIES

I. On Some Technical Procedures in the Use of the Einthoven Galvanometer

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IN outfitting for a new instrument it is often desirable, if for no other reason than that of economy, to make use so far as is possible of apparatus already at hand. Outfitting for the thread galvanometer admits of this practice to such an extent that it is thought worth while to report, among other things, a few of the devices that have been made use of in this laboratory.

The Main Switch-board

So complicated does the wiring become in setting up the apparatus for the thread galvanometer that instrument makers have found it profitable apparently to place the keys, metres, resistances, etc., with their proper connections, upon a table in a fixed position and offer the arrangement for sale as a single piece of apparatus.

The part of this, which we may call the main switch-board, as assembled in this laboratory, consists of the accessories necessary for the projection lantern, the storage of the electric bat-

teries and the current necessary to maintain and control the magnetic field in the galvanometer.

The accompanying diagram, Figure 1, shows the connections on this switch-board.

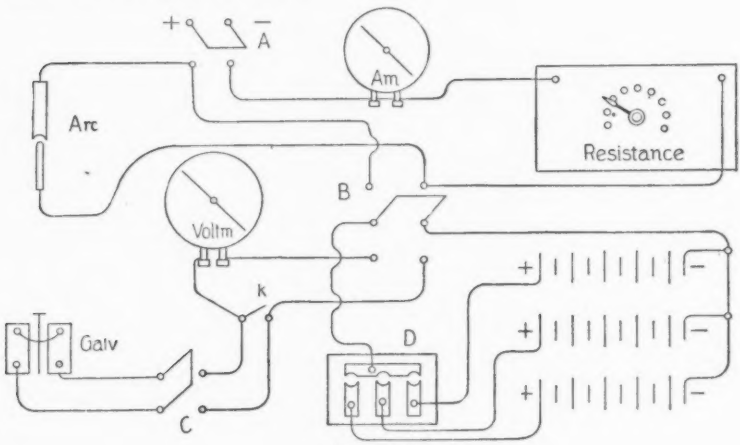

FIGURE 1

Of course the conditions of each laboratory will determine the exact disposition of the parts, many of which could be placed upon a board hung vertically upon the wall; other parts, such as the storage batteries, could be kept in some distant room.

In this particular case nearly all the parts shown in the diagram happen to be close together upon a table top and a shelf beneath. One end of the table abuts against the wall upon which are fixed the main key, A, and the ammeter. The storage cells of three batteries (five cells each) are placed upon the shelf under the table. The resistance box, capacity ranging from 0 to 50 amperes, must be near at hand on account of the need of constant adjustment. Upon the table top itself are placed the projection lantern, thread galvanometer, voltmeter, all the keys except A, and the wiring as shown.

Keys A, B, and C are double keys of the knife-edge type to be had of electrical supply houses, key B being a two-way key, and *k* a single key.

Key B may be regarded as the master key of the board. When closed in the upward position it admits of the charging of the storage cells. In this event the carbons of the lantern ("arc" in the diagram) are not in contact with each other and the resistance ("resistance" in the diagram) is set to supply a suitable amperage.

When key B is closed in the downward position the storage cells are available for the electro-magnet coils of the galvanometer.

By the wiring it will be noted that in no event can the main current supplied by A be put into communication with the galvanometer, and that key C, in a way, is superfluous. Its use (open) while charging the storage cells, however, is obvious.

Key D, a three-way plug key, admits of connecting any one of the three batteries of storage cells either with the main current for storing, or with the galvanometer for making the magnetic field.

The single key *k* by way of the voltmeter enables one to test the voltage of any one set of the storage cells at any time and independently of the galvanometer, that is, with C open.

The main key A may be supplied from the city's direct current, 110 or 220 volts. In case only an alternating current is available the lighting company may be induced to install a transformer at its own expense. In the latter event a rheostat interposed (also by the company) between the AC supply and the transformer enables one to adjust the voltage of the generating direct current within wide limits, say from 40 to 120 volts, a condition which is highly desirable in the many exigencies of experimentation.

The advantages of the adjustable coiled (dark) resistance, in the direct circuit after leaving A, are obvious when one remembers that one is working under the conditions demanded by photography. It is often desirable to "load" the batteries and the camera at the same time. This a lamp resistance would not easily allow.

The Photographic Registration Apparatus

The photographic registration apparatus so far supplied by the makers is apt to have the disadvantages of either a too narrow speed limit, or an unreliable, jerky movement. Both of these defects are particularly true of Edelmann's "Trommel-registrier Apparat." This apparatus, which I shall call the camera-drum in this paper, has been made very serviceable however by discarding the spring-motor supplied by the makers, and substituting, for the slower speed-ranges, the driving mechanism of a Baltzar kymographion.

A set of pulley wheels calculated to give several different speeds may be fixed to the axle of the kymographion. The pulley wheel on the friction disk of the camera-drum is too small and should be replaced by one of greater diameter, say 60 mm. One can thus obtain easily a half dozen steady speeds, of say 20, 30, 40, 60, 90 and 120 seconds per revolution of camera-drum.

For the rapid speeds which one finds necessary for graphic records of muscle-nerve latencies, for example, the Baltzar drum motor is not serviceable. But without any other change than that involved in changing pulley belts another device is employed. In this device a falling weight is used as follows.

A clutch of two pulley wheels as shown in the photograph, Figure 2, was constructed. One of the wheels carries the weights and rotates upon its axle at a fixed point; the other wheel revolves upon the same axle, and, when clutched to the first, transmits the motion by pulley belt to the camera-drum. But to facilitate the reversal of the weight-wheel and the setting of the camera-drum at its starting point, the transmission-wheel is movable along the axle so that it may be released from the weight-wheel at will. This latter movement is effected by means of a lever and ratchet device (shown in the photograph). When the lever is pulled down the transmission-wheel is drawn away from the weight-wheel against a strong spring which is wound spirally around the axle. The two wheels then are free to move independently of each other.

When the lever is released the spiral spring throws the transmission-wheel over against the weight-wheel, whereupon the two become locked by the clutches on their faces and must so rotate together as one wheel.

By varying the load of the weights, there being friction to overcome, one may obtain a variety of speeds. The fast speeds used so far in this work have been varied from one-half to two metres per second.

The demands of experimentation are such that a simpler driving mechanism cannot well be employed. For it is not only high speed one wants but the high speed must be developed

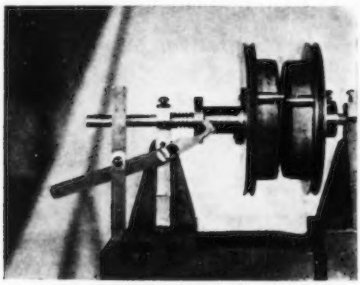

FIGURE 2. Photograph of clutch-device used for rapid speeds. (About one eighth actual size.) The transmission-wheel is held away from the weight-wheel by the lever, preparatory to reversing the weight-wheel and setting the camera-drum at its starting point. The wheels are then facing each other so that the clutch pegs fit into each other whenever the lever is released. The pulley belts and weights are not shown.

within one revolution of the camera-drum, — before the camera shutter opens and before the automatic stimulation signal-key (to be described directly) is thrown into action.

The "Fallapparat" of Prof. Max Cremer[1] is an ideal apparatus for rapid speeds, but the size of sensitized plate is limited to 15 or 20 cm. and for slow speeds one must again turn to another apparatus.

By using the devices here described one is able to employ the camera-drum for both slow and fast speeds by simply changing

[1] See Garten's article on photographic registration in Tigerstedt's Handbuch der physiologischen Methodik, p. 120.

a pulley belt. At the same time one retains the advantage of being able to use a sensitive surface 50 cm. long if needed. The power is also sufficient to operate simultaneously the spoke-wheel device of Garten for automatic ruling of the negatives.

An Automatic Stimulation and Signal Key

In muscle and nerve work, or in any work where a rapidly moving sensitized surface is required, it is obviously essential to have the stimulation key (whenever one is required) operated automatically by the revolving mechanism itself. By extending a lever arm from the stimulation key and allowing its end to fall in front of the camera shutter one may record photographically the exact point in time when the stimulation occurred.

In other words one has a stimulating key whose action can be controlled exactly as regards the time relations of the moving camera-drum, and at the same time a signal key that makes a record of this action with no greater latency than that of a beam of light travelling only a few millimetres.

The photo-signal key has been used before. The object here is only to describe the method of its application in the present series of experiments.

To the edge of the shutter of the camera-drum a specially shaped metal bar is fastened at about its middle point. The bar is free to move at this point and may be regarded as a lever of two arms. One arm extends a few millimetres over and in front of the slit of the shutter. Its end is flattened in the vertical plane and the edges shaped to give the sharpest image possible. We shall call this arm of the lever the signal arm.

The other arm of the lever extends beyond the edge of the camera-drum and is then bent at right angles backwards. Its end is flattened in the horizontal plane.

When the lever is set in nearly horizontal position this arm makes contact with the flattened end of a small rod extending downward in a fixed position. The two contact surfaces are of platinum and when actually in contact may complete an

electric circuit. We shall call this arm of the lever, therefore, the contact arm.

The contact arm is made to extend somewhat beyond the point of contact so that a stout finger fixed on the edge of the friction disk of the drum when in motion may strike it and thus break the contact automatically.

Since both arms are of one and the same metal bar and have the same fulcrum, a movement that strikes the contact arm down simultaneously strikes the signal arm up.

If, at the same time, a beam of light is falling upon the signal arm, and the camera shutter is open, the movement of the arm may then be photographed. The instant that the electric current is broken is thus recorded with as great accuracy and constancy as can be desired.

Summary

Certain technical devices and procedures are described for the equipment and operation of a thread-galvanometer in the usual physiological laboratory.

1. A description is given of a simple and perfectly safe disposition of the parts and wiring of an inexpensive main switchboard.

2. Certain modifications of Edelmann's photo-registration drum are briefly described which enable one to use it for both slow and fast speeds. The velocity of the camera-drum may thus be varied at will from 4 mm. to 2 metres per second.

3. A combined automatic breaking key and photo-stimulation signal is described which may be attached to the photo-registration drum.

The above devices have been used successfully, and thus at comparatively small expense, with the small electro-magnet thread galvanometer, Edelmann construction.

ELECTROMYOGRAM STUDIES

II. On the Time Relations and Form of the Electric Response of Muscle in the Single Twitch

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The Question

THE electric response of muscle to stimulation may have a significance for us in any one, or all, of its three essential features, namely, (1) its magnitude as measured in terms of electrical units, (2) its duration, as measured in units of time, and (3) its form by which we shall designate variations in potential and electrical sign which occur while the response continues.

The significance of any one, or all, of these features of the electric response, needless to say, is bound up with the general problem of the nature of muscular contraction. For a long time it has been generally conceded that the phenomenon is an expression of the excitatory process and its propagation in living tissue. Whether the act of contraction, in case of muscle, is itself in any way the direct result of the electrical change, has not been seriously considered by physiologists.

Furthermore the question as to whether the mechanical changes involved in the act of contraction give rise to, or are accompanied by, any one part of the electrical change also has apparently received little or no serious consideration.

And rightly. For the evidence favoring such views is both meagre and conflicting. Furthermore (it may be stated at the outset) no evidence has been obtained in the present investigation (when all the evidence is once rightly understood) which will support such views.

The evidence in the literature will be reviewed as briefly as one may and then compared with the results of the present investigation.

Historical

According to early observers the electrical response in muscle began with,[1] and culminated in the middle of,[2] the latent period, or at least preceded the contraction wave.[3]

Bernstein[4] by the use of his differential rheotome obtained results from which he could conclude that the negative phase of the electrical response was completed before the beginning of mechanical change (shortening).

Lee[5] using the capillary electrometer as early as 1887 observed that the duration of the change of potential may continue for the whole of the contraction period. Burdon-Sanderson[6] using the same instrument succeeded in photographing simultaneously both electrical and mechanical changes. He found that "the beginning of change of form in muscle and the culmination of the electrical change at the seat of origin may be synchronous." From his tabulated results it appears further that the electrical disturbance may continue at least during a part of the contraction.

Engelmann[7] did not believe that the electrical response had any direct relation to the mechanical process of contraction. In defence of his position he arrays a large number of facts and finally expostulates, "if the capillary electrometer shows the beginning of the electrical change to be later than, or syn-

[1] v. Bezold, A.: Monatsberichte der königlichen Akademie zu Berlin, 1861, pp. 1023, 1862.

[2] Helmholtz, H.: The same, 1854, p. 329.

[3] v. Köllicker und H. Müller: Verhandlungen der medizinischen Gesellschaft in Würzburg, 1856, vi, p. 528.

[4] Bernstein, J.: Untersuchungen über den Erregungsvorgang in Muskel- und Nervensystem. Heidelberg, 1871, p. 60.

[5] Lee, Frederic S.: Archiv für Physiologie, 1887, p. 204.

[6] Burdon-Sanderson, Sir J.: Journal of physiology, 1895, xviii, p. 148.

[7] Engelmann, Th. W.: "Über den Ursprung der Muskelkraft." Leipzig, 1895, p. 45.

chronous with, the change of form, then it shows it too late and thereby demonstrates its uselessness for the determination of such short time intervals." The chief objection lay in the experiments of Biedermann [8] who showed that muscle in certain stages of narcosis, when no longer able to show any trace of mechanical response, still gave well-marked electrical response to stimulation.

Samoijloff [9] again uses the capillary electrometer and with most beautiful technique photographs both electrical and mechanical responses.

He shows "that the greatest part of the electric process falls within the latent period of the muscle contraction. The rest of the curve is for the most part like that described by other authors." If one examines Samoijloff's curves (Figure 2, Plate I) one finds evidence that the electric disturbance continues after the beginning of contraction and even on into the relaxation phase.

After Einthoven introduced his thread galvanometer into physiology it was a natural wish to know what evidence this instrument could yield us in this field. Paul Hoffmann [10] made a study of the question and while he made no simultaneous records of mechanical and electrical changes, he states that the electrical disturbance in no case lasted longer than 0.08 second. "With good material the diphasic curve is all over in 0.01 second"; but, "in other cases, especially using smaller frogs, I found a great lengthening of the second phase."

Using the red and white muscles of mammals Arnt Kohlrausch [11] obtained practically the same results, the whole time of the electrical response being 0.03 and 0.015 seconds for the red and white varieties respectively.

Judin [12] had already published a photograph purporting to be the simultaneous record of both the movement of the galva-

[8] BIEDERMANN, W.: Sitzungsberichte der Wiener Akademie, 1888, xcvii, pt. iii, p. 101.

[9] SAMOIJLOFF, A.: Archiv für Physiologie, Suppl. Bd., 1908, p. 1.

[10] HOFFMANN, P.: Archiv für Physiologie, 1909, p. 489.

[11] KOHLRAUSCH, A.: The same, 1912, p. 283.

[12] JUDIN: Zentralblatt für Physiologie, 1908, xxii, p. 365.

nometer thread and the muscle lever in response to a single stimulus. In this photograph the chief electrical change appears to have occurred during the latent period but a considerable disturbance still seems to continue on into the period of contraction. Another remarkable thing about the curve is that it is complex, reminding one more of the curve described by Lee. If one ignores minor details the curve could be classed as being triphasic.

While there exists a great diversity of results concerning the duration of the electric response, the evidence concerning the form, with exception of that just noted, is much more unanimous.

When lead off from longitudinal and uninjured surface at certain points the form of the curve obtained is said to be generally, as Hermann [13] long ago described it, diphasic.

If one examines the photographic records however one finds the evidence not to be so unanimous as are opinions on this point. The capillary electrometer records for the most part show evidences of a third phase and Hoffmann speaks of a monophasic deflection occurring in uninjured frog's gastrocnemius, if the leading off electrodes are placed in certain positions on the muscle.

Hoffmann's work was especially directed toward clearing up the conflicting mass of evidence in this problem, and one may only regret that he did not include the mechanical changes in his records. From the line reproductions which are exhibited in the text, however, one may see that the galvanometer thread has not yet come to rest at what may be taken as the end of the second phase. The curves are not always pure monophasic and diphasic curves or even combinations of these.

To sum up then one may say:

1. While the electric response in muscle takes place for the most part during the latent period of the muscle contraction yet there is often a continuation of a slighter electrical disturbance after the muscle begins to contract. This may even be noted to persist during a part or all of the relaxation of the muscle.

[13] HERMANN, L.: Archiv für die gesammte Physiologie, 1878, xvi, p. 235.

2. When the muscle is led off from uninjured points the curve of the electric response is in the main diphasic. But often the return from the positive deflection (second phase) does not end at the null point but may pass beyond into a second negative phase, of small electromotive force, thus giving rise to an apparently triphasic response.

Are these irregularities which one finds so often in the records all due to experimental errors, to extraneous causes, and not at all to actual electrical changes arising within the muscle itself? The present investigation is an attempt to answer this question.

Experimental

The frog's gastrocnemius was used, since it has been the object of study in so many of the preceding investigations and also on account of its convenience. The muscle was stimulated by break induction shocks both directly and indirectly.

Both isotonic and isometric contractions were studied, special muscle levers having been constructed for the work. The isometric lever was carefully calibrated. While it was not perfectly isometric for the muscle yet at a tension of 500 gm. its writing point made an excursion of only 0.33 mm.

The small electromagnet model of Einthoven's galvanometer (Edelmann's construction) was used throughout.

The photographic apparatus and the special stimulation and signal key used are described in Part I of this series of studies.[14]

Results

The records being all photographs were carefully marked, corresponding notes were kept, and the analyses of the records tabulated. The features of these analyses bearing upon the problem of this paper are presented in the following protocols.

May, 1912. — The records of this month show the duration of the second phase (time from culmination of first phase to culmina-

[14] Snyder, C. D.: This journal, 1913, xxxii, p. 329.

tion of second phase) to vary from 7 to 68 thousandths of a second. In one set of experiments a third phase (negative) appears which comes to a culminating point at the end of about 100 thousandths of a second, or sigmata.

January, 1913. — The second phase requires from 7 to 42 sigmata to reach the culmination point. A slow third phase is likewise present in a few records.

February, 1913. — Time to culmination of second phase, 60 to 135 sigmata. No third phase remarked.

ANALYSIS OF PHOTO-RECORDS FROM THE EXPERIMENT OF JULY 19

Number of record	Mechanical Response				Electrical Response			
	Latent period	Shortening period	Relaxation period	Total time	Latent period	First phase	Second phase	Total time
Isotonic Contractions: 3								
8	7.5	65	52	125	3.84	3.84	4.8	12.48
	8.0	68	50	126	4.2	2.9	4.3	11.4
9	9.7	62	42	114	4.87	2.58	3.3	10.8
Average	8.4	65	48	122	4.3	3.1	4.1	11.5
Isometric Contractions: 4	15.0	46	31	92	3.81	2.72	4.5	11.03
5	14.9	47	30	91.9	3.33	3.3	4.4	11.03
6	12.7	48	32	92.7	4.11	2.62	4.67	11.4
Average	14.2	47	31	92.2	3.7	2.9	4.5	11.1

July, 1913. — Selected records of these experiments are here tabulated. They are typically diphasic. The thread used was of platinum with about 5200 ohms resistance, and a sensitivity of about 2.5×10^{-7} amperes for 10 mm. deflection at 75 cm. projection, a magnification of about 200 times, and a tension equal to that used in the experiments.

The deflections of the thread in the electric response of the muscles varied, for the first phase, from 2.0 to 8.3 mm.; for the second phase, from 2.2 to 13. mm.

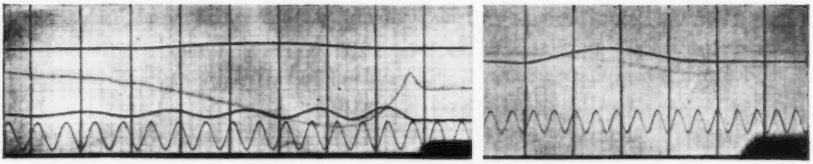

FIGURE 1. January 16, photograph No. 1. The second phase here, it will be noted, culminates only after the muscle begins to shorten. A third phase also appears culminating only after the contraction wave is passed. The muscle exerts a tension of about 100 gm. Leads from muscle, *a–c*.

FIGURE 2. April 2, photo. No. 3. The thread used has a resistance of about 7200 ohms. Stretch of nerve, 24 mm. Isometric lever. Leads, *b–c*. Resting current of about 4 mm. not compensated. Tension exerted by muscle, 200 gm.; actual shortening of muscle, about 0.13 mm.

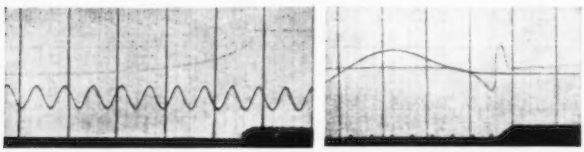

FIGURE 3. To show lag of thread upon break of direct current. Same thread at same tension as in Figure 2. Strength of current passing was about 0.01 milliampere Lag, 0.09 seconds. Deflection 13.5 mm.

FIGURE 4. July 13, photo. No. 1. Isometric lever. Tension produced by muscle, 350 gm. Thread used has resistance of about 5200 ohms. It will be noted that the second phase reaches culminating point at the moment the muscle lever begins to leave base-line.

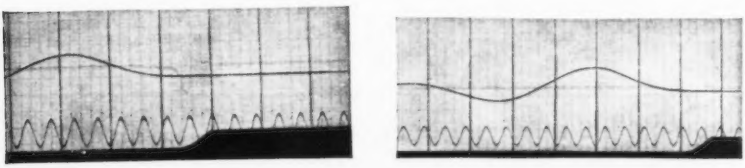

FIGURE 5. July 19, photo. No. 4. Same as in Figure 4 only the muscle lever rises a little later than the culminating point of the second phase of the electric response. The first slight deviation of the thread is due to a small amount of the stimulating current in the muscle-galvanometer circuit. This deflection coincides with the photo-stimulation signal below. Room temperature was about 28° C.

FIGURE 6. July 19, photo. No. 5. Same muscle as in Figure 5; same conditions. The lag in the thread is less in both Figures 5 and 6 than in Figure 4. This is due to difference in tension of thread.

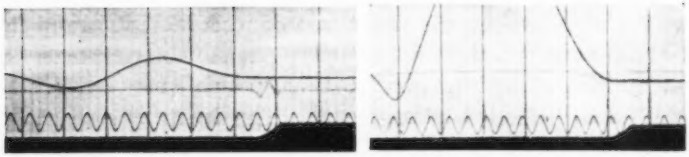

FIGURE 7. July 19, photo. No. 7. Same muscle and conditions as in Figure 6, save the direction of the stimulating current is reversed, which fact is shown in the record. The initial slight deviation of the thread is now downward instead of upward as in the other records. This proves it to be no part of the muscle response. The whole of the electric response is complete in the latent period!

FIGURE 8. July 19, photo. No. 3. Same muscle and conditions as in Figures 5, 6, and 7, save instead of the *isometric* an *isotonic* lever is attached to the muscle. The lever was loaded with a 40 gm. weight. The culmination of the second phase again falls synchronously with beginning of rise of lever. The muscle is changed in length freely; the deflection of thread at end of relaxation must be due to some movement of the leading off thread as also the slight irregularities at the beginning of shortening.

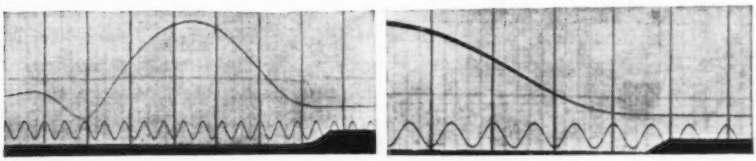

FIGURE 9. July 23, photo. No. 4. Same conditions as in Figure 8. Isotonic lever used. The hesitation of thread to return to null point at close of second phase and the slight deflection at end of relaxation must be due to slight displacement of the leading-off thread as in Figure 8.

FIGURE 10. July 23, photo. No. 1. Same muscle and lever as in Figure 9. The speed of the photographic film was greater than in the other photographs. In this record no stimulating current appears in the galvanometer circuit (see thread record).

The leads from the muscle were all from the $b-c$ position, that is, from the belly of the muscle at the neural equator and a point midway from that to the (achilles) tendinous end.

The length of nerve intervening between stimulating electrodes and muscle was nearly 40 mm. The preparation was at room temperature, which was about 28° C. The numbers in the table express thousandths of a second.

All the photo-records here reproduced except number 3 show simultaneous records of the galvanometer thread, the muscle lever, the vibrations of tuning fork (100 D. V.'s per second) and the stimulation signal. The latter is a lever key falling, upon opening the circuit, in the beam of light. The image of the thread is the dimmer line, that of the muscle lever the heavier, in the middle of the photographs. All records read from right to left. The leads from the muscle to the galvanometer are (unless otherwise indicated) the leads *b–c*. The figures are one half the original size.

The photo-records of experiments of July 12, 13, 16, and 23 all show data similar to that tabulated for July 19.

It is of interest to compare the figures of July 19 with those of Burdon-Sanderson [15] for frog's muscle stimulated indirectly with stretch of nerve of 12 mm., isotonic contraction. The times given are the actual times of each event:

Latency of response		Time to culmination of electrical response	
mechanical	electrical	1st phase	2d phase
8	4	5	4

The two methods of procedure which produced in the July experiments such different results from those obtained in the previous spring and winter are, (1) the use of a much tighter thread, and (2) the horizontal instead of the vertical suspension of the muscle.

Discussion and Conclusion

From the foregoing it appears that the electric change in muscle in response to single stimulus is, as generally accepted, purely diphasic in character. A third phase or any other deviation from the diphasic wave finds explanation either in uncontrolled stray currents, or in displacement of the leading off electrodes caused by the changing form of the muscle during contraction. If the muscle hangs vertically it is difficult to prevent the leading off thread from slipping (experiments in

[15] Burdon-Sanderson, Sir J.: *loc. cit.*, p. 148.

May, January, and February). Even where the muscle is suspended horizontally and every precaution is taken to keep the threads in position evidence of their displacement is still at hand in the isotonic twitch. (Figures 8 and 9.)

Of the pure diphasic curves we have two types, those which are completed within the latent period and those which are greatly prolonged into the contraction period. Of the latter variety whatever the conditions of the muscle or the position of the leads they are all stamped with a common character. The first phase is apparently of small E.M.F. and of short duration, the second phase is of relatively greater E.M.F. and of greater duration. Indeed the prolonged character of the response is due to the great duration of this second phase. The explanation of this kind of electric response is to be found in the system of the galvanometer used. If the swing of the galvanometer is too slow in giving expression to the full amount of current flowing such a curve may obtain. For if the system (in our case the thread) has not yet measured the full value of the first phase before the "negative charge" has reached the second electrode then it will be cut short in its deflection in the negative direction. Reversal of current has set in and it now swings in the positive direction. There being no more reversals of direction the system has time to take the full measure of the second or positive phase of the response. It is thus that we have curves of electric response in muscle with greatly prolonged second phases (see Figures 1 and 2).

Furthermore a loose thread after no current flows in it will *lag* in its return to the null position. (See Figure 3.) This is also a part of the "continued electrical disturbances" found in such curves as shown in Figures 1 and 2. The greatly prolonged curves obtained in the experiments of May, January, and February thus also find an explanation. The thread used at that time was much looser than in the later experiments.

Doubtless this fact will also account for the observations of others of a greatly prolonged second phase (Hoffmann's and Judin's for example).

The manner in which the leading off threads are slung around the muscle would also affect the character of the electric re-

sponse. If one thread lay along an exact cross-section, the other longitudinally for some distance or even diagonally, it is quite apparent how one phase would be of a greater duration than the other. Indeed three threads laid on the muscle, the middle one connected to one pole of the galvanometer, the other outer two to the other pole, would give a triphasic curve.

It is well known that the temperature coefficient of the velocity of the excitation wave in muscle is positive for a greater range of temperature than the temperature coefficient of the contraction time, and that that of the latent period of the mechanical response is greater than that of the latency of the electrical response.

Experiments made at different temperatures, therefore, would doubtlessly show some difference in the time relations of mechanical and electrical responses. But compared to the causes pointed out above this factor would be of slight value.

THE ENDURANCE OF ANEMIA BY NERVE CELLS IN THE MYENTERIC PLEXUS

By W. B. CANNON and I. R. BURKET

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RESULTS obtained by various investigators have shown that nerve cells of different classes and positions in warm-blooded animals show differences in sensitiveness to anemia.

The nerve cells of the cerebellum and cerebrum are most sensitive. Mayer stated that from 10 to 15 minutes was the limit of cerebral anemia, beyond which resuscitation is not practicable.[1] Batelli stopped the heart by the induced current and started it again after varying intervals; he found that the functions of cerebral cells were re-established if the blood supply was absent for only 10 minutes, but that after 15 minutes the restoration was no longer constant, and after 20 minutes it was impossible.[2] Results similar to these were reported by Stewart, Guthrie, Burns, and Pike.[3] Gomez and Pike, on examining the effects in detail, observed that the small pyramidal cells of the cortex were most sensitive — 8 minutes of anemia killed many of them; and that the Purkinje cells were next in order — 13 minutes producing in them diffuse chromatolysis.[4]

The cells of the medulla are more resistant. Gomez and Pike report that anemia for 8 to 13 minutes produces in them only slight lesions or no changes at all, and that anemia should last for 20 to 30 minutes in order to produce alterations incompatible with complete recovery. Stewart, Guthrie, Burns, and Pike describe one animal in which respiration returned

[1] Mayer: Medicinisches Centralblatt, 1878, xvi, p. 579.

[2] Batelli: Journal de physiologie et de pathologie générale, 1900, ii, p. 456.

[3] Stewart, Guthrie, Burns and Pike: Journal of experimental medicine, 1906, viii, p. 316.

[4] Gomez and Pike: Journal of experimental medicine, 1909, xi, p. 262.

after ligature of the innominate and left subclavian arteries for an hour, but in this instance there may have been a partial supply from below.

The cells of the spinal cord apparently withstand anemia for a somewhat longer period than those of the bulb. Ehrlich and Brieger produced permanent paralysis of the hind limbs, bladder, and rectum by clamping the aorta of the rabbit just behind the renal arteries from 45 to 60 minutes.[5] Spronck[6] and later Sarbó[7] demonstrated that anemia of the cord for an hour causes necrosis of all the nervous elements. Considerable variation in the effects of anemia was noted by Spronck; in one animal all symptoms of injury disappeared after the ligature had been applied for a half hour; and in another, recovery was incomplete when the blood supply was restored after 10 minutes. Sarbó states that the paraplegia disappeared in his experiments if the ligature remained for less than an hour.

The cells of the spinal ganglia are also resistant to lack of blood supply. Gomez and Pike found no sign of a pathological change in these cells when made anemic for 30 minutes.

Still more resistant are the cells of the sympathetic ganglia. Tuckett was struck by the very slight change in the nerve cells of the superior cervical ganglion when long deprived of their blood supply, but still surrounded with lymph.[8] Schröder applied more exacting conditions. He killed cats by narcosis and after varying intervals perfused the neck vessels from the carotid and tested the functioning of the cells of the ganglion by stimulating the cervical sympathetic trunk for its effect on the pupil.[9] Thus he succeeded in demonstrating a restoration of function when 60 minutes had intervened between the last breath and the beginning of perfusion. These, therefore, are the most stable nerve cells in the absence of blood supply that have thus far been described.

[5] Ehrlich and Brieger: Zeitschrift für klinische Medicin, 1884, vii, Supplement, p. 155.

[6] Spronck: Archives de physiologie, 1888, xx, p. 17.

[7] Sarbó: Neurologisches Centralblatt, 1895, xiv, p. 664.

[8] Tuckett: Journal of physiology, 1905, xxxiii, p. 79.

[9] Schröder: Archiv für die gesammte Physiologie, 1907, cxvi, p. 603.

Objects of this Investigation

Magnus has furnished evidence that rhythmic contractions of the alimentary canal do not occur if the myenteric plexus is removed.[10] And yet, as Mall has observed, a piece of small intestine may be removed from the body, kept on ice 24 hours, and on being perfused in a warm bath, will contract rhythmically.[11] If Magnus's evidence is correct the cells of the plexus must have continued living in the cold temperature for 12 hours or more after removal from their blood supply. This circumstantial evidence indicates that the cells of the plexus are exceptionally resistant to anemia, though of course the resistance could not reasonably be expected to endure as long in the warm body as in an ice-cold chamber. The hardiness of the intrinsic neurones of the alimentary canal, compared with those in other parts of the body, was the prime object of interest.

Lewandowsky has argued that Magnus's experimental procedure was faulty in that the plexus was removed by tearing the two muscular coats apart.[12] If by anemia the nerve cells are destroyed, a variation of the procedure is offered, and results thus obtained have an interesting bearing on the question of the neurogenic or myogenic origin of gastrointestinal contractions.

Learning the limits of endurance of anemia in these nerve cells is of interest also in relation to the possibility of continued functioning after the loss of blood supply in surgical states — as in hernia and intussusception.

For these different reasons the present enquiry was undertaken.

[10] Magnus: Archiv für die gesammte Physiologie, 1904, cii, p. 362.

[11] See Mall: Johns Hopkins Hospital Reports, 1896, i, p. 54. We have observed similar restoration of rhythmic activity when the intestines, kept ice-cold over night, was warmed in oxygenated Ringer's solution.

[12] Lewandowsky: Die Functionen des Zentral-Nervensystems. Jena, 1907, p. 92.

Methods

Two methods were used to produce anemia, — by ligature, and by compression.

The Ligature Method. — This method is illustrated diagrammatically in Fig. 1. The vessels supplying a zone in the stomach, and zones in the small and large intestines were tied, and the zones then shut off from neighboring parts by tape tied tightly about the canal. Zones thus isolated soon became purplish in color. By cutting the injected vessels on the surface of the canal it was possible to prove whether the blood supply had been completely excluded. Usually when the vessels of the colon and small intestine were cut no bleeding ensued. The blood supply to the stomach, however, was much more difficult to control by this means; and since gastric katastalsis is the most persistently rhythmic activity of the muscles of the alimentary canal, and the stomach, therefore, is an excellent indicator of the effects of anemia on contractions, the use of a more reliable method was desirable.

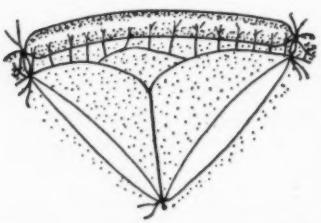

Figure 1. Diagram representing the method of isolating a segment of the alimentary canal by tying vessels and the canal itself. The mesentery is cut between the ligatures.

The Compression Method. — In producing anemia by compression the stomach or intestine was fixed tightly between two glass plates, with rounded edges and sufficiently long to project on either side of the flattened portion of the canal. The plates were pressed together either by rubber bands wound around the projecting ends of the plates or by screw clamps (see Fig. 2). The transparent glass permitted observation of the blood supply of the compressed parts and the pressure applied was always sufficient to render the tissues milky white. Perfect anemia was thus produced. A cut through the serosa and external muscular coat on either side of the glass plates, at the end of the period of anemia, left a mark which showed clearly the limits of the area which had been kept bloodless.

The periods of anemia lasted from 1 to 7 hours. During these periods the exposed parts were enclosed in a sheet of sterile rubber and kept warm. The operation was performed with careful asepsis. When the glass plates were removed a return of the circulation was in all cases ascertained by inspection. The animals (cats) were thoroughly anesthetized from the beginning of the operation until the abdomen was finally closed.

The Physiological Examination. — After a varying number of days following the operation the animal was examined for movements of the anemic part. On the pyloric end of the stomach or on the proximal colon, where continuously moving katastaltic or anastaltic waves can be observed with the X rays, activity was looked for by this means after food mixed with bismuth subcarbonate had beed fed. Failure of the waves to pass over the part which had been anemic would prove, of course, that the anemia had disturbed or destroyed the functioning of that part; whereas the uninterrupted progress of the waves would prove that it had had no effect.

On the small intestine the X ray method could not be employed satisfactorily; in order to test the activities of the gut after anemia, the animal was fed a few hours before being killed, and when anesthetized and on its way to death, the abdomen was opened and the intestine examined under normal salt solution at body temperature. Segmenting movements can be induced by tying the gut below any region of particular interest and permitting the contents to accumulate until there is increased internal pressure. This procedure was utilized to call forth activity in the anemic region of the gut. At the same time observations were made on the movements of the stomach and colon.

The Histological Examination. — After observations had been made to determine the presence or absence of activity in the parts which had been anemic, the animal was killed and these parts were excised and fixed in Bouin's or Zenker's fluid, or in acetic acid. They were then embedded in paraffine, sectioned serially, and stained with eosine and methylene blue. The microscopic examination was made with a Leitz ocular No. 3,

and with objectives 7 and $\frac{1}{12}$. Each cell studied was examined through the series in order to get the three-dimensional appearance.

Results

Physiological Examination. — In the earlier experiments in which anemia was produced by tying the vessels and the digestive tube, physiological tests soon showed that contraction was not destroyed within time limits far beyond the 60 minutes of anemia which nerve cells of the sympathetic ganglia will endure. In one of the first experiments four parts of the small intestine were isolated as shown in Fig. 1. In each place the main vascular trunks were tied with a linen ligature, and the anastomosis and the intestine itself tied tightly with tape. The procedure was carried out under ether anesthesia and with care for asepsis. At the end of a half hour all four isolated portions were purple and swollen, there was no pulsation in the vessels, the veins were full of dark blood, and yet there were some spontaneous movements of the gut. Removal of the ligatures of one portion at the end of a half hour resulted in prompt disappearance of the purple color, which was replaced by pink. The only difference from normal appearance was slight swelling of the region and minute subserous ecchymoses. As the blood reentered the region strong contractions occurred. The same changes occurred when the blood was readmitted to two other portions at the end of an hour. When circulation was restored in the fourth portion at the end of one and a half hours there were no spontaneous movements.

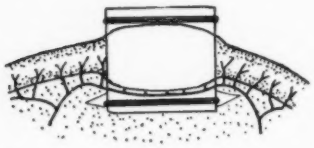

FIGURE 2. Diagram showing the method of producing anemia of a segment of the alimentary canal by including it between glass plates pressed together by rubber bands or screw clamps.

The absence of activity immediately after restoration of the blood supply in the last of the foregoing experiments does not mean that parts lose their functions by one and a half hours of such treatment. In Figs. 3 and 4 are shown waves passing over

the stomach and the colon; these parts had been tied off for two hours and at the end of that period were quite purple.

In another instance ligatures were placed on the stomach and proximal colon for *seven* hours. At the end of the period both parts were purple, but the stomach not so unnatural as the colon. Seventeen days later the movements of the canal

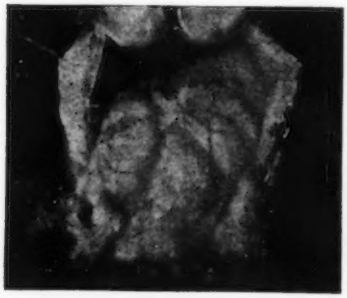

FIGURE 3. A photograph of katastaltic waves passing over the pyloric end of the stomach where the vessels had been tied for two hours.

FIGURE 4. A photograph of anastalsis in a part of the colon (between the threads) where the vessels had been tied for two hours.

were observed under salt solution. The stomach exhibited perfect katastalsis. The colon had ruptured and had been mended by the growth of other tissues about it, but the lumen was thus so much constricted that material could hardly be passed through.[13] In all probability the stomach in this case, in spite of its purple color at the end of 7 hours of "anemia," was not wholly deprived of blood supply. The results obtained under the more rigorous conditions of anemia produced by pressure indicate, indeed, that continuance of activity in this stomach

[13] In this instance the entire lower half of the small intestine was enlarged and thin-walled. Rhythmically-repeated waves of katastalsis, similar to those of the stomach, appeared here and there. A tonus ring made by applying a 5 per cent solution of barium chloride became a source of these waves; at times it caused no waves to pass downwards, but several to move upwards (anastalsis) for a distance varying from 2 to 4 cm. Thus the small intestine in the presence of obstruction had taken on some of the characteristics of the colon. (*Cf.* CANNON: American journal of physiology, 1912, xxx, p. 114.)

was possible only because some slight blood flow persisted, or because the presence of blood in the vessels, even though stagnant, keeps the tissues normal longer than they can be so kept in a wholly bloodless state. The limits of the endurance of this sort of anemia (after ligature) by the nerve cells of the myenteric plexus were not determined.

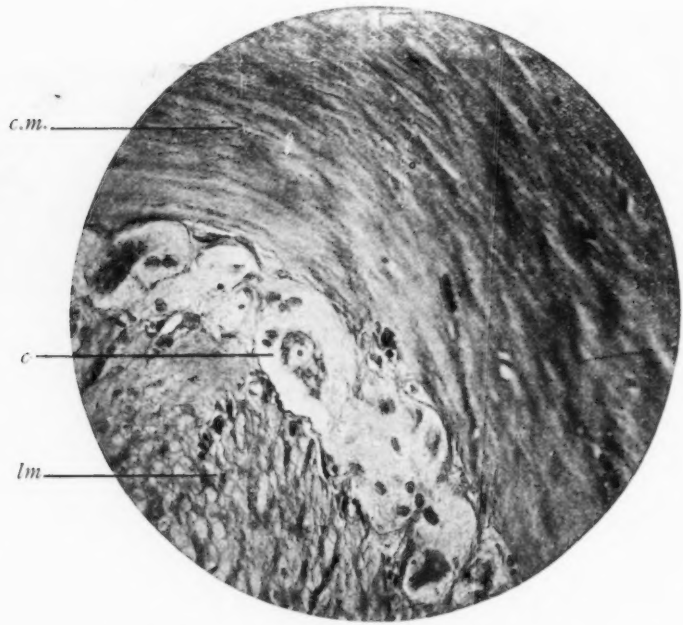

FIGURE 5. Section of a normal duodenum: *c*, nerve cell showing cytoplasmic granules; *cm*, circular muscle layer; *lm*, longitudinal muscle layer.

When complete anemia was caused by pressure between glass plates for any period up to 3 hours, and the gastric and intestinal contractions were examined later by the X rays or by inspection under salt solution, normal movements were invariably seen. In one instance perfect gastric katastalsis and occasional spontaneous constrictions of the proximal colon were seen, in regions which had been compressed for ***four*** hours. This result, however, was unusual. In all other instances compres-

sion for $3\frac{1}{2}$ hours was followed by failure of activity in the compressed region; and in three instances in which compression was applied for 4 or $4\frac{1}{2}$ hours perforation occurred and caused peritonitis. Complete anemia, with blood pressed from the vessels, can persist, therefore, at body temperature for about 3 hours, without destroying the ability of the alimentary canal to contract normally.

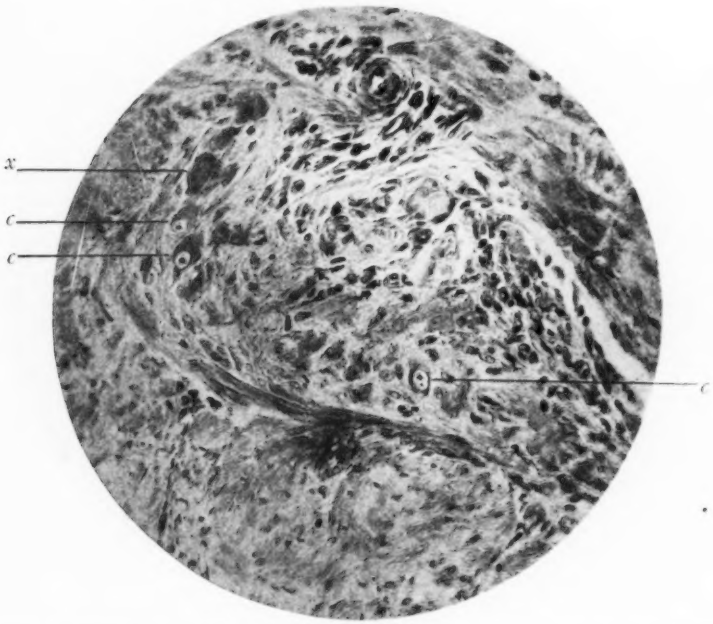

FIGURE 6. Section of a part of the canal which had been compressed for three hours, showing a nest of nerve cells with moderate connective tissue proliferation. *C*, *c*, *c*, nerve cells cut through different levels; *x*, section of a nerve cell passing only through cytoplasm.

Histological Examination. — In the tissues rendered anemic by tying vessels no degeneration of nerve cells was observed even when the anemia had lasted for as long as 6 hours. As already stated, however, there was some question as to whether the anemia in these cases was complete. These results, therefore, are chiefly valuable in showing that the nerve cells may

remain normal even if they are in a part of the stomach or intestines which has been for many hours deprived of its normal circulation, and is edematous, and purple with stagnant blood.

In tissues compressed till white for 1, 2 and 3 hours the nerve cells in the compressed region usually appeared quite normal.

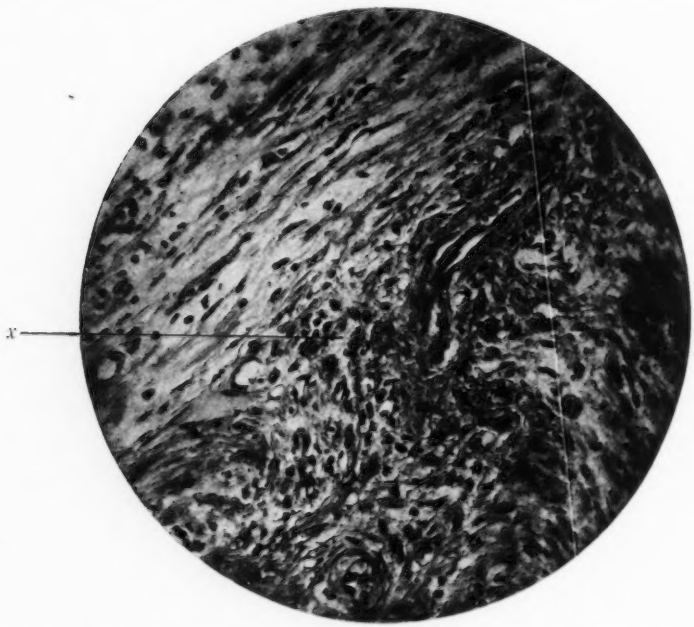

FIGURE 7. Section of a part of the duodenum which had been compressed three and a half hours. Specimen taken sixteen days afterwards. Muscle tissue partly degenerated, infiltrated with round cells, and to some degree replaced by connective tissue. *X*, original site of myenteric plexus between the muscular layers; nerve cells absent.

Comparison of the appearance of the cell bodies of the myenteric plexus in a normal intestine (see Fig. 5) and in a region under pressure for 3 hours (see Fig. 6) shows that when changes occur they consist in a disappearance of the cytoplasmic granules, a diffuse staining of the cytoplasm, and a moderate connective tissue proliferation. The nuclei become somewhat eccentric.

In the majority of cells anemic for 3 hours changes were not observed.

As a rule, in parts of the alimentary canal compressed $3\frac{1}{2}$ hours or longer practically all nerve cells disappear from between the two muscular layers (see Fig. 7); only occasionally can degenerated remnants be found. The muscular tissue also undergoes marked changes, — becoming infiltrated with round cells and in part replaced by connective tissue.

Summary and Conclusions

If the vessels supplying parts of the alimentary canal are tied and the parts themselves limited by ligatures, the tissues become edematous and purple, but may not be completely anemic. After the condition has persisted for 6 or 7 hours such regions may recover normal activities and on being examined histologically show nerve cells with normal appearance.

If complete anemia of parts of the alimentary canal is produced by compression between glass plates, the condition may last for as long as 3 hours without loss of normal motility or of nerve cells in the compressed regions. If the compression-anemia lasts for $3\frac{1}{2}$ hours or longer, it almost invariably results in loss of function and disappearance of nerve cells in the compressed parts.

The concomitance of persistence or loss of function with persistence or destruction of nerve cells in these experiments supports Magnus's contention that the spontaneous contractions of the alimentary canal are of nervous origin.

The continued existence of the cells of the myenteric plexus after 3 hours of complete anemia — 2 hours longer than the cells of sympathetic ganglia — reveals them as the most hardy nerve cells thus far found in the body.

EVIDENCE OF FAT ABSORPTION BY THE MUCOSA OF THE MAMMALIAN STOMACH

By CHARLES W. GREENE and WILLIAM F. SKAER

[From the Department of Physiology and Pharmacology, Laboratory of Physiology, University of Missouri]

VON KÖLLIKER discovered evidence of the absorption of fat from the gastric mucosa in mammals in 1857.[1] He said, "I have never failed to find fat in the gastric epithelium of young cats, dogs, and mice." "The content of the cells varies greatly from the finest fatty granules scarcely perceptible to the condition of the cells in which they were strutting with the engorged fat." This discovery together with the discovery of the fat splitting enzyme lipase by Claude Bernard in 1856,[2] and the discovery of the cleavage of fat in the gastric cavity by Marcet in 1858[3] marks the historical beginning of the small and scattered literature on the subject of gastric fat absorption. That fat is absorbed through the intestinal mucosa was shown by Weber in 1847.[4]

The newer methods for the histological identification of fat lead us to take up a re-study of this important problem with the hope of settling some of the questions which have arisen in the last few years concerning the gastric absorption of fats.

Method in Outline

Our method of observation rests on the determination of the microscopic variation in the quantity of fat in the gastric tissues in relation to fat feeding. In brief it is as follows: we fed ani-

[1] Kölliker, A. Von: Verhandlungen der physicalisch-medicinischen Gesellschaft, 1857, vii, pp. 174–193.

[2] Bernard, Claude: Leçons de physiologie expérimentale, 1856, ii, p. 178.

[3] Marcet, W.: Medical Times, 1858, xvii, p. 209.

[4] Weber, E. H.: Archiv für Anatomie, Physiologie, und wissenschaftliche Medizin, 1847, vi, pp. 400–402.

mals with a constant diet to secure relative stability of conditions, then withheld food for a definite time, usually 24 hours. After this time a meal rich in fat and of the desired character was given. Very rich cream was used in the later experiments for testing the absorption of fat. For the rats olive oil was mixed with fresh ground meat. Young animals were allowed to suck the mother's milk where possible, but occasionally were fed artificially. As a rule several animals have been run in parallel, each group selected of the same litter or as nearly the same age as possible. Animals were chosen of different ages from those that had never taken food to adults. The animals were killed by anesthetics at varying times following digestion and absorption. The stomach was opened and its undigested content carefully removed and the mucosa rinsed with luke warm physiological saline. Selected tissues were fixed in 10 per cent formalin for about two hours, then sectioned by Bardeen's freezing microtome, stained with saturated alcoholic-alkaline scarlet red, counterstained lightly with haematoxylin, mounted in glycerine, and sealed. We find these preparations keep very well for a few weeks, depending largely upon the care with which traces of alkali are removed after the staining with scarlet red.

Results of Observations

Our observations may be divided into two groups, namely,

1. The evidences of fat absorption by the gastric superficial epithelium.

2. The variation in fat content of the mammalian gastric glands.

1. The Evidences of Fat Absorption by the Gastric Superficial Epithelium

The study of frozen sections cut vertical to the free surface chosen from the two principal regions of the stomach, namely, the cardiac and the pyloric, reveals a rich absorption of fat by the superficial epithelium. In both regions the quantity of fat and its distribution in the cells bears a definite relation to the

time that has elapsed since fat feeding. The assumption that the fat observed is a true absorption fat rests upon the constancy of this cycle in its relation to fat feeding. This cycle was shown long ago for the intestinal epithelium by Eimer.[5] Eimer observed that in the small intestine and in the upper part of the large intestine the epithelial cells of the mucosa were filled with fat droplets first in the outer, i.e., superficial, zone, and that later the basal portion of the cell filled with fat.

The normal comparison for such a series of experiments would seem to be a fat free epithelium. As a matter of observation, however, it is extremely difficult to secure an absolutely fat free epithelium, a fact that was also observed on the intestinal epithelium by Eimer. The most favorable condition for comparison is that represented by the fasting stage of from 20 to 24 hours. At this time the gastric epithelium still contains a few fat droplets usually at the deeper, i.e., basal, ends of the cells. For our purposes this condition was taken as a standard.

After a fat meal is given to a series of 24-hour fasted animals and these are killed at successive periods of time extending through a range of, say, 3, 6, 10, 15, and 20 hours, it is noted that the superficial epithelial cells load with fat droplets to a maximum in from 6 to 15 hours. The loading is first in the free ends of the cells, i.e., the surface bordering on the lumen. The fat droplets gradually increase in number and size until they occupy all the space between the free surface and the nucleus. At the most superficial margin of the cell the fat droplets are always extremely small, usually a fraction of a micron. In the zone surrounding the nucleus the droplets are always larger. In both regions of the gastric epithelium the appearance gives one the impression that the fat droplets are growing larger by accretions as they pass from the marginal zone toward the nucleus. At this stage in very young animals the contrast is not so sharp as regards the size of the droplets as in older animals. As time progresses the fat droplets appear in ever increasing numbers in the basal end of the cell, i.e., between the nucleus and the basement membrane. At this period the entire epithelial

[5] EIMER, TH.: Virchow's Archiv für pathologische Anatomie, 1867, xxxviii, p. 428.

cell is often engorged with fat, the droplets varying in size from a fraction of a micron to as much as 5 or 6 micra in diameter. The total mass of fat that may appear in an epithelial cell in that maximal stage of fat absorption seems to vary according to two factors. The first and most important of these is the factor of the age of the animal. The younger the animal apparently the greater the mass of fat that will be crowded into the cells at this stage of the cycle. The second factor is that of the quantity of fat that is being digested. We are convinced that the greater the mass of fat present in the lumen of the stomach in an active stage of digestion the greater the quantity in the cells at the maximum of the absorption cycle. In relation to intestinal absorption various men have called attention to the fact that the passage of fat is a progressive one, that, while fat is being absorbed into the free surface of the cell it is being lost or discharged from the basal end of the cell. Evidently this is also true for the gastric mucosa, so that a balance at any given instant must exist as between the quantitative amount of dissociated fat in the lumen, and consequently in the absorbing cell itself, and the quantity of synthesized fat appearing in droplets within the cell.

As absorption ceases the free ends of the cells begin to lose fat until very little is present between the free surface and the nucleus. This disappearance of fat is indicated by two microscopic factors, namely, a diminution in the absolute size of the fat drops and a decrease in the number present. Cells in which the free margins are relatively low in fat content as a rule show only small sized fat droplets. This stage is still characterized by a heavy loading of fat droplets in the bases of the cells, i.e., between the nuclei and the basement membrane. As a final stage, 24 hours or more, the basal portion of the cells lose the fat droplets until only liposomes are left in the extreme basal ends of the cells, the condition which was taken as the normal.

There is one slight variation in the above cycle, namely, the fact that at the very beginning of fat digestion and at the time just before the appearance of fat droplets in the superficial ends of the epithelial cells the normal remnant of fat in the bases of

the cells tends to disappear. In other words, there is a distinct but slight drop in the fat content curve just at the beginning of fat digestion. This drop, which we found very difficult to interpret at the beginning of our series of experiments, proved to be easily explained on the assumption of an increased quantity of lipase which is undoubtedly secreted at this time. The increase in lipase is sufficient to hydrolize the fat remnant, thus sending it into solution, in which condition we of course could not find it by our fat staining methods.

Comparison of Cardiac and Pyloric Absorption. — The evidence of fat absorption is strong in both the cardiac and pyloric regions. A comparison of the two regions in one and the same animal generally shows a slight contrast in the relative quantity of fat in the most superficial cells. One must remember here that the crypts of the cardiac end of the stomach of most mammals are small and insignificant in comparison with the wide open funnel-like crypts of the pyloric mucosa. It is probably for this reason that the epithelial cells that load with fat in the cardiac end are only those representing the areas between the crypts. Unless absorption is very rapid and voluminous the cells in the mouths of the cardiac crypts rarely contain more than traces of absorption fat. On the other hand, the most superficial mucosa of the pyloric region is always in relatively intimate contact with the semi-fluid digesting mass. These cells are the ones that are so often engorged with fat. The size of the droplets is somewhat larger than in other portions of the stomach and occasional areas are observed in which the cells seem actually to be distended from the excess of internal pressure. In the pyloric region the cells lining the crypts, especially near the mouth, show a considerable loading with fat. Traces of fat are found even down relatively deep in the cells of the crypts.

The cycle of variation in fat quantity and in its distribution in the cell is the same whether one considers the cardiac or the pyloric mucosa.

Comparison of Gastric Fat Absorption in Different Animals. — As previously stated, we have used rats, cats, and dogs as experimental animals in this study. Of the last two species we have examined animals of various ages from birth to the adult.

In the cats we have made comparisons between the young just before taking nourishment and after the first sucking. In each of these classes fat absorption occurs in the gastric regions. The stomach of the rat near the esophageal opening is lined with stratified epithelium. This area was not shown to absorb fat. Our examinations were limited to two adult animals, a number entirely insufficient to determine the point. Both the cardiac epithelium and the pyloric were loaded with fat in the rats we examined. These rats were fed olive oil mixed with ground meat and cooked in small sausages. The control rats showed only the normal small quantities of fat at the bases of the epithelial cells.

In dogs, especially in the young puppies, the fat fed animals present an unusually heavy loading of fat in the epithelial cells. The fat droplets were of relatively large size and in quantity sufficient to engorge the most superficial regions.

In cats the mucosa is composed of smaller cells than in dogs. However, the loading of fat during absorption runs a close parallel with that in dogs. Our series of animals of different ages was run on cats. Just born kittens which have taken no nourishment show extremely fine liposomes in practically all the gastric tissues — the mucosa, gland cells, muscle coat, etc. The liposomes have a characteristic fine granular, evenly distributed appearance, but were not excessive in amount in any of our material. Kittens from the same litter that were allowed one meal gave a beautiful picture of fat loading in the gastric superficial epithelium. The picture is so characteristic and so readily obtained that this one experiment is sufficient to dispel any doubt as to the source of the fat. Adult cats reveal fat absorption by the gastric mucosa. Other things being equal, the quantity of fat is much less in the adult than in the very young. However, we must take exception to Weiss' statement [6] that the power of fat absorption is lost by the gastric mucosa at an early age.

Considering the series of animals studied it seems that the power of fat absorption by the gastric mucosa is greater in the

[6] Weiss, Otto: Archiv für die gesammte Physiologie, 1912, cxliv, pp. 540-543.

young than in the adults, a factor which has been discussed by Schilling.[7]

2. The Variation in the Fat Content of the Mammalian Gastric Glands in Relation to Fat Absorption by the Stomach

An important observation running through this series of experiments is found in the fact that the amount of fat in the gastric glands increases through a definite cycle following a fatty meal. In some cases the gland cells are so filled with fat as to practically obscure the histological structure. The fat is generally distributed throughout the protoplasm of the cell, always exclusive of the nucleus. In the peptic glands fat granules appear first and most strikingly in the parietal cells, later in the chief cells. In the pyloric glands the fat appears first and in greatest quantity in the cells at the bottom of the glands. In cats and in dogs the pyloric glands are often convoluted at their bases not unlike though less marked than in the sweat glands. The fat in the cells of these convolutions when stained with the scarlet red brings the glandular areas into sharp contrast with the supporting tissues.

After a meal the fat is loaded into the gland cells relatively slowly, and in like manner is extremely slow to disappear. The maximum amount of fat in the gland after a fatty meal does not occur so soon after fat feeding as in the case of the superficial epithelium. In some cases this maximum appears as late as 20 to 24 hours. During fasting the fat in the cells disappears even more slowly, often taking several days to reach a stage which one would consider as comparatively free of fat. Taking it all in all, the cycle of variation in the fat content of the glands runs its course much more slowly and does not show such extremes of fat content as in the case of the epithelium. However, the variation is definite and quite adequate to be easy of identification. The length of time involved seems to us to be the factor which has led certain observers, notably Nikolaides[8] to consider fat in the glands of the body as degeneration fat. However, we are con-

[7] Schilling, F.: Allgemeine Wiener medicinische Zeitung, 1901, xlvi, pp. 279, 291, 301, 313.

[8] Nikolaides, R.: Archiv für Physiologie, 1899, pp. 519–524.

vinced that the condition that we have had under consideration is one definitely and directly dependent upon the amount of fat passing through the walls of the alimentary tract during absorption. There is no evidence of protoplasmic degeneration.

Last April Schickele [9] published a paper in which he uses the term "Wanderfett" to designate the fat that accumulates in the tissue organs such as the liver, sweat glands, etc., during certain conditions. This term might well be applied to the case of excessive fat in the gastric glands observed in our work.

The fat content of the glands, like that of the epithelium, also undergoes a drop at the beginning of the digestion period. We have come to the conclusion that this depression of fat in the glands is directly related to physiological change occurring in the early stages of fat digestion, a fact which is readily explained on the view of the reversible reaction of lipase given us by Kastel and Loevenhart [10] and by Loevenhart [11] himself.

When food reaches the stomach there is a great increase in the gastric secretions, owing to the usual normal reflexes. This must now, in light of the work of Marcet, Volhard,[12] Laqueur,[13] and others, be accepted as a time of great increase in lipase produced by the activity of the gastric glands. The effect is that there is an increase in the normal lipase content not only of the gastric gland cells, but of the other adjacent structures of the mucosa also. The net result is that the normal fat of the gland cell is suddenly surrounded with an increase in the lipase. This disturbs the balance of the fat remnants in the gastric glands and in the mucosa as well, which, according to the Shütz-Borissow law, should lead to a further dissociation and disappearance of

[9] SCHICKELE: Verhandlungen der deutschen pathologischen Gesellschaft, 15th session, 1912, pp. 451–454.

[10] KASTEL and LOEVENHART: American chemical journal, 1900, xxiv, p. 491.

[11] LOEVENHART, A. S.: This journal, 1901, p. 331.

[12] VOLHARD, FRANZ: Münchener medizinische Wochenschrift, 1900, pp. 141, 194.

Ibid: Zeitschrift für klinische Medizin, 1901, xlii, p. 414.

Ibid: Zeitschrift für klinische Medizin, 1901, xliii, p. 397.

[13] LAQUEUR, ERNST: Beitrage zu chemischen Physiologie und Pathologie, 1906, viii, pp. 281–284.

these fat remnants. The very process, therefore, of the production of lipase for the digestion of the fat content of the canal leads to a local disturbance of fat equilibrium in the gastric glands, a disturbance which involves also the gastric epithelium.

With the beginning of absorption of the digested fat there is first a loading of the fat into the columnar epithelial cells, later a diffusion of this fat into the membrana propria, lymphatics, gland cells, etc., with a final reconcentration of the fat into the gland cells. It is obvious, therefore, that the increase in content of fat in the gastric gland cells takes place somewhat later in the cycle of fat absorption than the increase noted in the columnar epithelial cells.

3. The Variation in Absorption Fat in Relation to Lipase Action

Throughout our work we have accepted Pflüger's hypothesis that fat digestion and absorption is a lipolytic process. By this theory lipase dissociates the fat in the canal and the dissociation products can pass the surface boundaries of the epithelial cells. Kastel and Loevenhart's demonstration of the reversibility of lipolytic action is adequate to account for the reappearance of stainable fat in the tissue cells. The quantitative amount of fat is an expression of the balance as between fatty acid and lipase, a proposition which may also be stated in the reverse way. This hypothesis adequately explains the histological picture of fat variation observed in our experiments. If one applies here the Shütz-Borissow law, according to which the quantity of the cleavage fat is proportional to the fourth power of the cleavage agency, it is obvious that we have a working hypothesis into which fit very nicely the observed facts not only as to the relative quantity of fat, but also the gross quantity in both the absorbing epithelium and in the glands.

In establishing our normals we studied a series of fastings of various durations. In occasional animals of medium to late fasting duration we found an unexpected quantity of fat in the various gastric glands. Such cases would be ordinarily judged as fatty degeneration, but the character and arrangement of the fat in the cells and the otherwise normal appearance of the tissues was not that of the typical and unquestioned pathological de-

generation. It seems rather to be a normal condition. We soon came to the conclusion that we were dealing with a disturbance as between lipase production and fat mobilization, i.e., a case of Schickele's "Wanderfett." In the early stages of fasting the labile substances, namely, the carbohydrates, are quickly used for the production of energy. A little later the fats of the adipose tissues and other more fixed substances are drawn upon. In this later stage the lipase producing tissues are doubtless strongly stimulated to increased activity for the accomplishment of the transportation of fats. Lipase was proven by Loevenhart to be a normal content of a large number of tissues of which certain glandular tissues are particularly mentioned by him. To these tissues ought to be added the gastric glands which are lipase producers. If one assumes that an excessive production of lipase takes place in these glands at the time during fasting when the fats are being dissolved from the storage tissues and are present in a relatively high per cent in the circulating fluids, it follows that there will be an increased synthesis of fats in the lipase producing tissues themselves. We believe from our evidence that the gastric glands are of this class. This hypothesis more adequately explains the histological picture shown in our second case than does the hypothesis of fat production from degeneration of the cell protein.

In conclusion, let us emphasize the main contribution in our paper, that *there is a definite cycle of variation in quantity of fat in the gastric mucosa and in the different gastric glands in relation to the time following a meal rich in fats.*

Summary

In a series of experiments based on the study of the amount of fat in the superficial gastric epithelium and in the gastric glands at different times in relation to feeding and fasting we have come to the following general conclusions:

1. In puppies and kittens before the young have fed, the young gastric tissues show minute stainable granules which have the histological characteristics of fat. These granules are extremely small and are characteristic in their distribution.

2. The gastric epithelium of rats, cats, and dogs shows a definite cycle of variation in fat content following the taking of a meal rich in fat.

3. The gastric epithelium in a moderately fasting condition contains traces of fat in the bases of the cells. This condition has to be taken as the normal in fat absorption experiments.

4. The epithelial fat content following a meal of fat is expressed by a curve which at first shows a slight drop, then a rapid rise of the amount of fat in the cells, followed by a more gradual and slow decline to the normal.

5. The gastric epithelium and gastric glands of puppies and kittens both peptic and pyloric show a marked increase in the stainable fat after the taking of the first meal. Under ordinary conditions this fat is never reduced to the quantity and characteristic arrangement of that in the embryo.

6. The amount of fat in the gastric glands runs a definite and characteristic cycle of variation in relation to the taking of fatty foods. This cycle is marked by an initial slight drop with a slow and prolonged rise to a maximal and fall to the normal. The extremes of the fat content of the glands vary much less than in the epithelium.

7. More fat is found in the pyloric than in the peptic glands and the contrasts are more pronounced in the pyloric glands.

8. It is difficult to cause the entire disappearance of fat from the mammalian gastric gland cells by prolonged fasting.

9. In medium to late fasting there is occasionally an increased quantity of fat in the gastric gland cells. This has no relation to absorption fat but is explained as due to mobilization of body fats.

WILLIAM FREDERICK SKAER died June 16, 1913. His loss is deeply felt by his associates, who saw in him the promise of a most useful life.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

VI. A Study of the Mechanisms of the Hunger Contractions of the Empty Stomach by Experiments on Dogs

By A. J. CARLSON

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I. Experimental Procedure

THE contractions of the empty stomach in dogs were recorded by means of a bromoform manometer connected with a delicate rubber balloon in the stomach. The balloon was introduced into the stomach either through a gastric fistula or through the esophagus. We were surprised to learn the ease with which a small rubber balloon and rubber tube attachment can be passed through the esophagus into the stomach in dogs. If gentle dogs are selected for the work and the dogs are handled gently, they make little or no resistance after the first two or three experiments. I have never observed vomiting or gagging in dogs as a result of the introduction or the presence of the stomach tube in the esophagus. On the contrary, the dog with the rubber tube and balloon in the stomach and esophagus will lie quietly for hours in the lap of an attendant, while the tonus and movements of the empty stomach are being registered on the kymograph. Frequently the dog will go to sleep during the experiments. This is especially the case if the dog is covered up with a coat or a comforter. The tube in the esophagus does not cause distress or inhibition of the stomach movements.

Most of the observations were made on dogs with a fistula in the fundus of the stomach. In our first dog we made use of

the classical silver cannula. In all the other dogs we discarded the metal cannula and adopted the method followed in human gastric fistula cases. The incision (3–4 cm. in length) is made 3 cm. below the last rib and 5–6 cm. to the left of the linea alba. The oblique and transverse muscles are carefully separated without cutting them. The desired region of the gastric fundus is pulled out through this opening. The peritoneum is sutured to the fundus pouch. The abdominal muscles are similarly sutured to the pouch. In making these sutures care is taken not to penetrate deeper than the muscle layers of the pouch. The apex of the fundus pouch is then slit open, and the edges sutured to the edges of the skin. A closed rubber tube of 1 cm. in diameter is passed through the opening into the stomach and kept in place for four days. Then the tube and dressing are removed. It is found that the abdominal muscles compress this narrow pouch to such an extent that there is virtually no leakage from the stomach, much less leakage in fact than even in the most successful fistula using the metal cannula. There is no trouble in way of closing up of the fistula as long as the animal is being used two or three times a week. The dog takes care of the slight leakage, so there is no corrosion of the skin. We have dogs now in the laboratory with such fistula of six months' standing, and the dogs are in the best of condition. In fact, it is obvious that this fistula leaves the stomach much more normal than does the silver cannula method. We have obtained normal hunger contractions of the empty stomach thirty-six to forty-eight hours after making the fistula. Nothing like normal hunger contractions is seen in the stomach for six to ten days after making the fistula by means of the metal tube.

The splanchnic nerves were sectioned through an incision in the linea alba. The splanchnic nerves on both sides were therefore cut in one operation.

The vagi nerves were sectioned in the chest. We found the following method most serviceable. The incision (5 cm. in length) is made on the left side between the eighth and ninth ribs and well toward the back. The intercostal muscles are cut midway between the two ribs leaving the pleura exposed for a

distance of 5 cm. All this can be done with practically no bleeding. The pleura is then sectioned, the esophagus with the adjoining vagi hooked up by an aneurism needle, pulled up to the chest opening, and the vagi sectioned 3–5 cm. above the diaphragm. This can be done and the chest closed in less than three minutes, so that artificial respiration (by means of a tracheal tube) is not necessary. It is best to use artificial respiration, however, as that diminishes the pneumo-thorax and in some cases there may be some delay in picking out the vagi. The animals make rapid and uneventful recovery.

In the beginning of this work the animals were kept suspended in comfortable hammocks during the observations on the gastric hunger movements. It soon became apparent, however, that any kind of mechanical restraint on a young, vigorous and very hungry dog causes restlessness and evident distress, especially when continued for hours. Training will overcome this in part, but not completely. Distress and restlessness will obviously interfere with the stomach movements. We therefore tried the expedient of having an attendant keep the dog snugly in his lap during the observation period. This proved very satisfactory, except for the attendant. It is irksome, to say the least, to sit still for two to eight hours at a stretch. We can appreciate the reason for the dog's restlessness when restrained mechanically in a hammock or on a couch for that length of time. When the attendant knows how to handle dogs even a very hungry dog will lie in his lap quietly for hours, and will usually cuddle up and go to sleep. After a few experiments most dogs seek the research room by preference, and jump into the attendant's lap voluntarily. Two dogs became so well trained that they would lie quietly on a pillow for two or three hours at a time without any restraint whatever. It is obvious that mental stress and restlessness interferes with the stomach contractions not only in the way of direct inhibition but also by the varying tonus and irregular contractions of the abdominal muscles.

The animals used in these experiments were mostly young and vigorous females.

II. Results

1. *The character of the motor activities of the empty stomach*

The contractions of the empty stomach, as registered by means of a delicate balloon in the fundus, fall into three types according to the degree of tonus of the stomach.

Type I. — When the stomach shows feeble tonus the hunger contractions show an average duration of about thirty seconds,

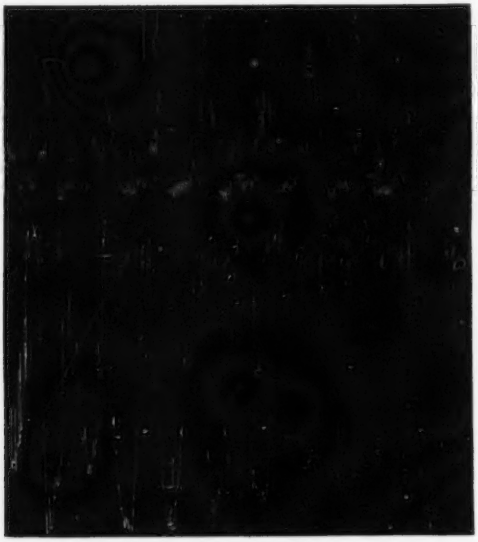

Figure 1. (One half the original size.) Tracings from the empty stomach (fundus) of dogs. Bromoform manometer. *A*, slow and feeble hunger contractions of Type I; *B*, rapid and vigorous hunger contractions of Type II.

and the intervals between the contractions vary from half a minute to three or four minutes. This type of contractions usually falls into groups, separated by intervals of relative quiescence. The duration of the groups vary from half an hour to three hours, and the number of contractions in each group varies correspondingly. It is very rare that a contrac-

tion group of Type I ends in the tetanus so frequently observed in man (Mr. V.). The group usually begins with feeble contractions but of longer than average duration and relatively far apart, and the contractions become gradually stronger and the intervals shorter. The end of the group is usually characterized by contractions of gradually decreasing strength. A typical tracing illustrating this type of the hunger contractions is reproduced in Fig. 1A.

Type II. — When the stomach is in relatively strong tonus the hunger contractions follow one another in rapid succession,

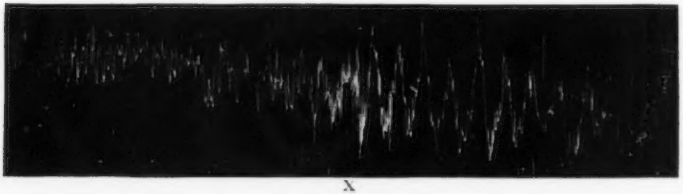

FIGURE 2. (One half the original size.) Record of hunger contractions of the empty stomach of dog. At *X* the gastric contractions change from Type III (incomplete tetanus or strong tonus) to Type II.

that is, without any intervening pause. The duration of the contractions varies between twenty and thirty seconds. These contractions are frequently interrupted by periods of incomplete tetanus lasting from one to five minutes. These periods of tetanus are practically identical with those previously described in man. The contractions of this type do not fall into distinct groups. They may vary to some extent in amplitude and rate, but otherwise may be continuous for an observation period lasting from two to six hours. If the animal becomes restless during the observation period the hunger contractions become irregular and may cease altogether, but this is probably due to splanchnic inhibition, and cannot be regarded as a spontaneous cessation of the hunger contractions.

This type of hunger contractions seems to be present only in young and vigorous individuals in excellent physical condition. Similar contractions were observed in our man, but less frequently than in our young and vigorous dogs. From obser-

vations on Mr. V. it is certain that the hunger sensation is practically continuous during these contractions. A tracing illustrating this type of the hunger contractions is reproduced in Fig. 1B.

Type III. — The hunger contractions designated as Type III constitute virtually an incomplete tetanus of the stomach. This tetanus is characterized by periods of strong and relatively persistent tonus on which are superimposed a series of rapid contractions. The duration of these rapid contractions averages twelve to fifteen seconds. These contractions are evidently analogous to the twenty seconds rhythm in man (Mr. V.). These tetanus periods vary in length from one minute to ten minutes. In prolonged starvation they may last much longer. In moderate hunger they are interspersed between groups of the Type II rhythm as shown in Fig. 3.

FIGURE 3. (Four fifths the original size.) Record of hunger contractions of the empty stomach of dog. At *x* dog allowed to see and smell meat. Showing inhibition of the hunger contractions through the splanchnic nerves.

This description of the gastric hunger contractions in dogs is based on observations on twenty-four individuals. The shortest observation period on each animal was two weeks, the longest five months with records taken, on the whole, every third day. The data should therefore be typical. The three types of contractions may be observed in the same dog on different days, or Type I may obtain for a few days, and then be superseded by Type II, etc. As a general rule Type I predominated in some of the dogs, and Types II and III in others. Some of the tracings also disclose what may be termed *transition stages*. Thus, near the end of a contraction period of

Type I the rapidity of the contraction may approach that of Type II, and occasionally the individual contractions of Type II will for short periods slow up to such an extent that they parallel Type I. This is to be expected, since the types of the hunger contractions seem to vary with the degree of gastric tonus, and this tonus may vary considerably during a single observation period. It is also to be noted that the hunger contractions may occasionally be feeble, irregular or practically absent for at least two to four hours at a time in dogs that are *seemingly* in good condition. And this is always the case if the dogs are in poor condition from any cause. One of the dogs developed pneumonia (fatal), and three of the dogs serious nose and eye infections. The dog in pneumonia showed an hypotonic stomach and complete absence of the hunger contractions. The dogs with the nose and eye infections showed very feeble and irregular hunger contractions during the period of infection.

The credit of discovery of the rhythmical contractions of the empty stomach in dogs belongs to Boldireff, but Boldireff's account of this rhythm is incomplete and partly misleading. According to Boldireff the contractions always come in groups of twenty to thirty minutes duration, and during the one and one half to two and one half hours intervals between these groups the stomach is completely quiescent. The contractions observed by Boldireff were evidently short and feeble periods of the Type I contractions, but the duration of the interval between the contractions given by Boldireff is on the whole much greater than that shown in my series. Boldireff evidently never obtained the Types II and III rhythm in his animals. The difference in the results of Boldireff and our own are probably due to (1) the condition of the animals, (2) the method of handling the animals, and (3) the method of registering the stomach contractions. Boldireff used the classical silver cannula for the gastric fistula. This depresses the stomach. All of his dogs had in addition to the gastric fistula (fundus) also duodenal, pyloric, pancreatic or hepatic fistulæ. His dogs were therefore subjected to much greater disturbance of digestion and metabolism than is the case of a simple fistula of the fundus as prepared by me. The dogs not being in the best of

condition, it is not surprising that they showed only feeble rhythm of Type I. But it seems likely that forcing the dogs by mechanical means to lie or stand in one position for six to twelve hours at a time is also partly responsible for the brevity of the contraction periods and the length of the intervening periods of quiescence. It is my experience when dogs thus treated become restless, and restlessness always is accompanied by gastric inhibition, probably through the splanchnics. When the dog is allowed to make himself comfortable in the lap of an attendant he lies quietly and usually without any restraint. This condition is certainly more nearly normal.

The tracings published by Boldireff do not show the respiratory intragastric pressures, nor do they indicate the slightest variations of the gastric tonus during the observation periods. His method of registration was therefore not delicate enough to detect small variations in the intragastric pressure. It would seem, however, that his method ought to have recorded the Type II contractions, if they had been present in his dogs.

2. *Reflex or Psychic Inhibition of the Hunger Contractions*

Anything which interests, annoys, frightens, or angers the dog causes temporary inhibition of the hunger contractions (Fig. 4B). Cerebral processes of pleasant character such as the entrance of a friend (animal keeper) into the room cause almost as marked inhibition as fear or anger. Pain seems to cause the most pronounced and lasting inhibition, but the *hunger pain* itself is an exception.

Particular attention was given to the influence on the hunger contractions of seeing and smelling palatable foods. It was noted in a previous communication that seeing and smelling palatable foods when hungry did not seem to affect the gastric hunger contractions in our man V. Neither did the sight or smell of food induce contractions in the quiescent stomach. *In dogs the sight and smell of food leads to temporary inhibition of the hunger contractions, and the inhibition is directly proportional to the degree of interest taken in the food.* This is a true reflex or psychic inhibition, because it appears too quickly and is too

temporary to be an acid inhibition (psychic secretion of gastric juice) from the stomach mucosa. The inhibition from the sight and smell of food is most marked during the first few tests in each dog. Most dogs soon learn that they are not to be allowed to eat the food. Dogs thus "sophisticated" pay little or no attention to the food shown them, no matter how intense the hunger contractions, and when this is the case the sight and smell of food does not influence the hunger contractions. This particular inhibition is therefore of the type of "conditioned"

FIGURE 4. (One half the original size.) Typical records of the gastric hunger contractions of dogs with section of the splanchnic nerves (*A*) and the vagi nerves (*B*).

reflexes (Pawlow) similar to the "psychic" secretion of gastric juice on seeing and smelling palatable food. These reflexes not only require the presence of hunger and appetite processes, but also a certain fixation of the attention on the foods, and this is apparently not possible when the dog knows that he is merely being teased with the food.

In a few instances the sight and smell of food seemed to induce a few contractions in the quiescent stomach. This is, indeed, the reaction that I expected to find to the sight and smell of food. Some such cases have also been observed in our

man V. It seems that we have the nervous mechanism for such a reaction in the motor or tonus fibres of the vagi. But since this motor effect on the stomach is so rare and transitory I hesitate to conclude that the motor neurones to the stomach via the vagi can be stimulated by the sight and smell of palatable foods. I am rather inclined to think that the few exceptions to the usual inhibitory reflex are coincidences. Few irregular contractions are frequently seen during the period of relative quiescence of the stomach. In the dogs another source of error appears in the sniffing, the altered respiration, and the altered tension on the abdominal muscles, when food is smelled or seen. All these changes affect the intragastric pressure. In our man V. a few forced respiratory movements induces a strong contraction even in the quiescent stomach, probably through associated tonus innervation via the vagi.

The mechanism of the reflex or psychic inhibition of the hunger contractions described above have now been partly determined. When the splanchnic nerves are cut on both sides, these inhibitions are greatly diminished and frequently absent. The main efferent path is therefore via these nerves, either through inhibitory fibres to the stomach wall or through the secretory fibres to the adrenal glands causing hypersecretion of adrenalin. The inhibition appears too quickly to be initiated by adrenalin, but the latter may be a contributory factor. The slight degree of inhibition that may be obtained after section of the splanchnic nerves must come about either through impulses through the few inhibitory fibres in the vagi acting on the stomach wall, or (more likely) through inhibition of the vagus tonus; that is, a central inhibition.

When this phase of the work was outlined the possibility was recognized that section of the splanchnics may change the above inhibition to stimulation, by eliminating the bulk of the inhibitory nerve fibres to the stomach. I anticipated that after the section of the splanchnics the sight and smell of food would initiate or augment the gastric hunger contractions by increasing the vagus tonus. But I failed completely to substantiate this hypothesis. The gastric tonus mechanism via the vagi is there and unimpeded by inhibition via the splanchnic

nerves, but the quality and quantity of the afferent nervous impulse so far tested seem to act on this tonus mechanism only in the way of inhibition.

It has been shown in our man V. that the stimulation of nerve endings of taste and general sensibility to the mouth cavity causes temporary inhibition of the gastric hunger contractions. Even the chewing of palatable food inhibits the contractions. While the experiments on Mr. V. are conclusive for man, I attempted to repeat the tests on dogs, as I surmise that these inhibitions from the mouth cavity are characteristic for the whole vertebrate series. This phase of the work on dogs did not yield satisfactory results. To be sure, stimulation of nerve endings in the mouth by gustatory substances and the chewing of palatable food leads to temporary inhibition of the hunger contractions of the stomach in our dogs. But these measures also cause salivation, swallowing movements, restlessness, and struggling. We have seen that the mere sight and smell of food suffices to cause inhibition. Swallowing causes temporary inhibition, and this is invariably the case with restlessness and struggling caused by discomfort, displeasure, or anger, or anything of unusual interest. All these factors are easily controlled in man, but they cannot be controlled in dogs.

3. *The Influence of Sleep on the Gastric Hunger Contractions*

It was hoped that the gastric hunger contractions during sleep would shed some light on the question of central innervation (via the vagi) of these contractions, as the condition of sleep seems to involve diminished activity of the cerebrospinal system. Lombard showed that even the light sleep of few minutes duration in the middle of the day depressed or abolished the knee reflex. I have studied the gastric hunger movements during similar short periods of light sleep in our man V., the man going to sleep in the course of an experiment while reclining in an easy chair or lying on a couch. In no case did I observe any effect of these periods of sleep on the intensity or rate of the hunger contractions. These contractions invariably continued as if not influenced by the change in the

central nervous system accompanying sleep. These short periods of sleep so far studied probably never reached the degree of central depression that is characteristic of the normal and uninterrupted sleep during the night. I have not yet succeeded in observing the hunger contractions in Mr. V. during a normal night sleep, for the reason that the substitution of the rubber balloon for the six o'clock dinner does not seem to be conducive to sleep, in his case.

When a dog is lying comfortably in the lap of an attendant, and is covered up with a gown or a comforter, he frequently goes to sleep for periods varying from a few minutes to an hour or more, provided the hunger contractions are not too intense, and the room is kept fairly quiet. These periods of sleep are characterized by the usual change in respiration, pulse, and muscular tonus, and not infrequently by snoring. During these periods of sleep the gastric hunger movements persist unchanged both as regards rate and intensity. In case the dogs are restless or disturbed in some way before going to sleep the hunger contractions usually become stronger and more regular during the sleep. This is obviously due, not to an increased tonus innervation through the vagi, but to the cessation of inhibition processes through the splanchnics. If very strong hunger contractions appear during the sleep the dogs invariably become restless, even to the point of "moaning" in their sleep, and usually wake up. The waking up always causes a temporary inhibition of the hunger contractions. On waking up during a period of relative quiescence of the empty stomach the action of stretching and the increased tonus of the skeletal muscles is frequently accompanied by what appears to be a temporary increase in the gastric tonus, and sometimes even a few hunger contractions. This may be an instance of associated tonus innervation through the vagi, but the mechanical effects on the stomach of the increased tonus of the abdominal muscles must also be taken into account, and eliminated through section of the vagi. It may be noted in this connection that our man V. never has gone to sleep during the experiment at the period when the hunger contractions were moderately strong or very intense.

It is admitted that the above observations on man and dogs probably do not include the state of deepest possible normal sleep. But the results show that light sleep has no effect on the rate and intensity of the gastric hunger contractions, except in the way of removal of extrinsic inhibitory influences via the splanchnic nerves. If the gastric hunger contractions are initiated or augmented by tonus impulses through the vagi this central tonus mechanism is not materially influenced by the change involved in light sleep.

On the other hand, the gastric hunger contractions of the empty stomach do affect the sleep. It is a common experience that the healthy individual experiences difficulty in going to sleep and sleeps less soundly on an empty stomach, provided he is not extremely fatigued, when we probably have to do with a direct depression of the stomach by fatigue substances in the blood. The dog is actually aroused from his sleep by the intense hunger contractions of the stomach. It is highly probable that this also occurs in man, especially in infants and in young and vigorous individuals. This is due to action on the vaso-motor centres and on the reflex centres in general by the afferent impulses from the walls of the contracting stomach. Whether or not an individual is awakened from his sleep by the gastric hunger contractions is simply a matter of the algebraic sum of the intensity of the hunger contractions and the intensity of the sleep.

The origin of the afferent impulses that cause the hunger sensation has been demonstrated to be in the walls of the stomach. But in case any person should want additional proofs, it may be pointed out that the above observations furnish additional evidence that the sensation or consciousness of hunger does not originate the gastric hunger contractions, since these contractions go on unchanged during sleep. This conclusion may be objected to on the ground that our man V. as well as our dogs might have been *dreaming* of hunger, food, etc., during these periods of sleep. In the case of Mr. V. there were no such dreams, at least none that he could recall when questioned immediately after he woke up. And as for the dogs, we have as yet no means of finding out, except by sectioning

the vagi. The gastric hunger contractions do persist after isolation of the stomach from the central nervous system. But it is not unlikely that a person awakened from his sleep by strong hunger contractions of the stomach will dream of hunger, food, etc., during the process of regaining full consciousness.

4. The Influence on the Gastric Hunger Contractions of Isolation of Stomach from the Central Nervous System

(1) **Complete Section of the Splanchnic Nerves.** — Observations have been made on five dogs with complete section of the splanchnic nerves on both sides. The longest period of observation after the splanchnic section was two months. Observations were in some cases begun two hours after the operation. When the records of these five dogs are viewed as a whole, it is clear that the *complete section of the splanchnic nerves in dogs increases the gastric tonus and augments the gastric hunger contractions* (Fig. 5A). The hunger contractions become more rapid and more continuous, that is, there is less evidence of the periodic groups with intervening periods of relative quiescence. It is not uncommon to observe contractions at the rate of about two per minute during an entire observation period of two to four hours. The section of the splanchnic nerves does not abolish the periodicity completely, however. It seems to be a question of relative degree of gastric tonus. If for any reason the tonus of the empty stomach is relatively low on any day, the hunger contractions are less frequent, and there is greater evidence of periods of relative quiescence. We desire to emphasize the fact that the above conclusion is based on the observations as a whole. Even the dogs with the splanchnic nerves sectioned showed on some days no greater tonus of the empty stomach or greater rate and persistence of the gastric hunger contractions than does the dog with these nerves intact. And occasionally a dog with the splanchnic nerves intact exhibits as great a degree of gastric tonus and rate and persistence of the gastric hunger contractions as the maximum observed in dogs with the splanchnic nerves cut. This is to be expected, as by section of these nerves one eliminates only one (and in

the normal animal probably one of the least important) of the factors in the motor activity of the empty stomach. The conditions that effect the stomach through the blood, and through the vagi are still subject to the same variations as in the animal with the splanchnic nerves intact.

After complete section of the splanchnic nerves the psychic or reflex inhibition of the gastric hunger contractions already described is greatly diminished. The stimuli that cause anger,

FIGURE 5. (Two thirds the original size.) Record of exceptionally vigorous hunger contractions of the empty stomach of dog thirty days after complete section of the vagi and the splanchnic nerves (isolation of the stomach from the central nervous system). This record was taken on the fifth day of starvation.

fear, pain, joy, or pleasure no longer lead to complete cessation of the hunger contractions. The maximum effect is a slight and transitory weakening of the contractions. It is therefore evident that the inhibitory fibres in the splanchnic nerves (and possibly also the secretory fibres to the adrenals) constitute the main efferent path in this type of inhibition. The slight degree of inhibition usually in evidence after section of the splanchnic nerves must be due to central inhibition of the vagus tonus or to action of the few inhibitory fibres in the vagi.

Particular attention was given to the effect of seeing and smelling food on the hunger contractions in these dogs with

section of the splanchnic nerves, in order to determine whether these stimuli augment the tonus of the vagi and thus increase the hunger contractions. The results were negative. Even with the greater part of the extrinsic inhibitory fibres to the stomach eliminated, the sight, smell, and taste of food not only fails to initiate or augment the gastric hunger contractions, but so far as these stimuli affect the stomach at all it is in the direction of inhibition of the hunger movements. The apparent increase in the intensity of the hunger pangs in man on seeing or smelling palatable food must therefore be essentially a central phenomenon of facilitation or Bahnung.

(2) **The Section of the Vagi.** — Section of both vagi in the chest was made in three dogs, and after this operation observations on the gastric hunger contractions were continued for from two weeks to three months. Observations were in some cases made two hours after the vagi section.

Section of the vagi leaves the empty stomach on the whole permanently hypotonic, that is, at least for a period of up to three months after the operation. The tonus of the empty stomach in these dogs varies somewhat from day to day, and occasionally the tonus may approach that of a dog with the vagi intact, but on the whole the tonus is permanently much lower than normal. This is evident not only from the observations by means of the balloon in the gastric cavity, but also on direct inspection and by palpation (introducing the finger through the fistula).

The hunger contractions of the empty stomach are changed mainly in rate and regularity. The duration of each individual contraction is about normal, or on the whole less than normal. The long-drawn-out contractions or tetanus are rarely seen. But the intervals between the contractions vary on the whole from two to five minutes or even up to eight minutes. The strength or rather the amplitude of the individual contractions may appear greater than normal, evidently because the contractions start rather suddenly and without any marked preliminary increase in tonus, and the maximal contractions are evidently so complete that all the air is forced out of the balloon. These contractions may continue of fairly uniform

amplitude and rate for two to three hours, that is, during a whole observation period. (Fig. 5.) The contractions vary in strength and rate from day to day, and on some days they may be completely absent during the entire observation period (two to four hours).

The periodicity of the hunger rhythm is, on the whole, obscured, except on the days when the gastric tonus approaches that in normal dogs. On such days the contractions appear at shorter intervals, and tend to fall into groups similar to those in normal dogs. Periods of gastric hunger contractions of normal rate and intensity have been observed as early as twelve hours after the complete section of the vagi in the chest. The period of most powerful hunger contractions so far observed in any dog was recorded in one dog twenty-four hours after the vagi section. This dog had during the four weeks preceding the vagi section showed almost invariably the Type II rhythm. It was therefore a dog with unusual intense gastric motor activity. The complete section of the vagi causes on the whole less depression in dogs that exhibit great hunger contractions while the vagi are intact. The variations in the rate and intensity of the gastric hunger contractions in different dogs is therefore primarily due to individual variations in the condition of the stomach rather than to variations in the central innervation or the central inhibition.

In the dogs with the vagi sectioned, but the splanchnic nerves intact, the "psychic" or reflex inhibition of the gastric hunger contractions is still in evidence, but the inhibition appears not to be so marked as when the vagi are intact. Accurate comparisons are, however, difficult to make because of the lowered tonus, and the usual long intervals between the hunger contractions after section of the vagi. I had expected an augmentation of the inhibition through the splanchnics after the vagi section. Instead of finding this to be the case there actually appeared a *gradual diminution in the influence of the splanchnic nerves on the empty stomach in the dog observed for three months after section of the vagi.* It was not due to the regeneration of the vagi fibres, and consequent restoration of the vagus tonus. If further work should establish this as a

fact, we would have a significant instance of physiological readjustment — either an actual diminution in the inhibitory impulses through the splanchnics in consequence of a dynamic readjustment in the central nervous system, or else an increased resistance ("tolerance") to the splanchnic impulses on the part of gastric motor mechanism.

5. *Section of both Splanchnic and both Vagi Nerves*

Complete section of the splanchnic and the vagi nerves were made on four dogs, and observations made on the gastric hunger contractions for thirty to sixty days after the observation. The section of the splanchnic nerves were made seven days after the section of the vagi. After this complete isolation of the dogs' stomach from the central nervous system, there is practically a permanent hypotonus of the stomach except under conditions of prolonged starvation. The gastric hunger contractions are much the same as when the vagi alone are severed. The contractions are usually of great amplitude, but the intervals between the contractions are frequently longer than in normal dogs. The grouping of the contractions into periods is usually in evidence. These contractions of the isolated and empty stomach are present ten to twenty hours after the vagi section, and there is some improvement in the rhythm or an approach towards the normal tonus and contraction rate during the thirty to sixty days of observation. On the whole the hunger contractions of the isolated stomach conforms to Type I. The Type II is rare except during prolonged starvation. Short periods (two to three minutes) of incomplete tetanus are frequently seen especially during prolonged starvation, and during the first half of the hunger period. It is therefore clear that *all the essential characteristics of the hunger contractions of the empty stomach are determined by the local gastric motor mechanisms* rather than by the character of the central innervation or the central inhibition.

Cannon[1] has reported observations on the effects of vagi and splanchnic section on the gastric movements of digestion

[1] CANNON: This journal, 1906, xvii, p. 429.

in cats. Section of the splanchnic nerves did not affect the movements of digestion; section of the vagi caused slowing and weakening of the peristalsis of digestion, but the normal rate of peristalsis was practically restored in a few days. Combined vagi and splanchnic section left the digestion movements of the stomach practically normal even shortly after the operation. It seems that section of the vagi or complete section of the vagi and the splanchnic nerves in dogs cause on the whole a greater change in the movements of the empty stomach than does the same lesion in cats in case of the movements of the

FIGURE 6. (One half the original size.) Tracings from the empty stomach of dog *A*, dog normal and showing normal hunger contractions; *B*, after dog developed pneumonia, showing complete absence of the hunger contractions.

filled stomach. This probably means that the tonus of the vagi plays a greater rôle in the movements of the empty than in the movements of the filled stomach. For it is not likely that there is such marked difference in the relative importance of the vagi in cats and dogs.

The changes in the character of the gastric hunger contractions after isolation of the stomach from the central nervous system seem primarily due to the persistent hypotonus. This is indicated by the fact that on days when the stomach of a normal dog shows relatively slight tonus, the hunger contractions approach the type shown by the isolated stomach, and

on days when the isolated stomach exhibits tonus approaching that in normal dogs the hunger contractions tend to assume the normal type. Occasionally records are obtained from the empty and isolated stomach that practically demonstrate the above point. During a period of relatively slow hunger rhythm the tonus for some unknown reason may increase markedly for periods of varying length *and during these periods the hunger contractions are identical in rate and character with those of the intact stomach in normal (strong) tonus.* In one of the dogs with the vagi and splanchnic nerves sectioned, six days fasting led to the appearance of periods of very great gastric tonus and during these periods (virtually periods of incomplete tetanus) the gastric contractions assumed the form of Type III.

However, the details of the changes in the hunger rhythm after isolation of the stomach from the central nervous system seem of minor importance in this connection. The essential point is that *since the empty stomach completely isolated from the central nervous system does exhibit the typical hunger contractions, the primary stimulus to these contractions is not to be sought in the extrinsic nerves.* This type of activity is characteristic of the empty stomach and primarily independent of the central nervous system. We do not mean to deny that under certain conditions the tonus fibres in the vagi may inaugurate or intensify the gastric hunger movements. But it seems likely that *under normal conditions the essential rôle of the vagi and the splanchnic nerves in connection with the gastric hunger contractions is that of modifying or regulating a primarily automatic mechanism in the stomach wall.* Further analysis of the hunger mechanism must be directed primarily to the intrinsic neuromuscular apparatus of the stomach, and secondarily to the factors that control the vagus tonus.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

VII. The Inhibitory Reflexes from the Gastric Mucosa

By A. J. CARLSON

[From the Hull Physiological Laboratory of the University of Chicago]

I. The Problem

IT has been shown by experiments on man (Mr. V.) that weak acids (including normal gastric juice) and alkalies, alcoholic beverages (wines, brandy, beer), coffee, tea, and even water acting on the gastric mucosa inhibit the gastric tonus and the gastric hunger contractions.[1] This inhibition is initiated by stimulation of nerve endings in the gastric mucosa, and not by mechanical tension or pressure on the stomach wall. The work on man led to the conclusion that any substance capable of stimulating the nerve endings in the gastric mucosa causes inhibition of the tonus and the hunger contractions, and inhibition only, as there was no evidence of any increase in the gastric tonus or hunger contractions following the primary inhibition.

The possibility that these inhibitory phenomena in man are in reality psychic inhibitions (consciousness of being subjected to experimentation) and hence not initiated by the stimulation of nerves in the gastric mucosa was considered. The following facts seemed to speak against the hypothesis of central or psychic inhibition. (1) The inhibition is proportional to the quantity and concentration of the material introduced into the stomach, and frequently Mr. V. did not know either the nature or the amount of the substance introduced. (2) The inhibition appears even when Mr. V. himself puts the wine or beer of his own

[1] Carlson: This journal, 1913, xxxii, p. 245.

choice into the stomach. (3) Mr. V. was at no time impatient or displeased with the experiments so far as I could determine. (4) These substances produce identical inhibitions when put directly into the stomach of dogs (awake or asleep). (5) The inhibitions are in evidence after isolation of the stomach from the central nervous system by section of the vagi and the splanchnic nerves. The present paper deals with the character of this inhibition in dogs and the modification of the inhibition following section of the splanchnic and the vagi nerves. For an account of the special technique and experimental procedure in this work the reader is referred to the previous reports.[2] It will suffice to state that liquids were introduced into the stomach through the fistula by means of a soft rubber tube so that swallowing acts, and the stimulation of nerve endings in the mouth, the pharynx, and the esophagus were completely eliminated. These tests on dogs are thus parallel to those on Mr. V.

II. The Action of Water, Acids, Alkalies, and Alcoholic Beverages

The observations were made on six dogs with all the extrinsic gastric nerves intact, on six dogs with the splanchnic nerves cut; and on four dogs with complete section both of the vagi and the splanchnic nerves. The results on the normal dogs are practically identical with those on Mr. V. already reported. Gastric juice (human, canine), weak acids and alkalies, brandy, wines, and beer introduced directly into the empty stomach during hunger contractions produce immediate inhibition of the gastric tonus and contractions. The duration of the inhibition depends upon the quantity of the material introduced into the stomach and on the degree of the hunger contractions. Thus the same quantity of gastric juice or wine seems to cause more prolonged inhibition in dogs showing the "type I" than in the dogs showing "type III" hunger rhythms. The duration of these inhibitions can best be studied in the dogs showing the type II and III hunger contractions, as these two forms are practically con-

[2] Carlson: This journal, xxxii, p. 369.

tinuous, so that the errors from spontaneous periods of relative quiescence are eliminated. In normal dogs showing type II and III contractions, 25 cc. gastric juice or 0.5% HCl usually causes complete inhibition for 20 to 30 minutes. The return of the hunger contractions is always gradual. 25 cc. of beer will inhibit for 15 to 25 minutes. In one case 50 cc. of beer caused complete inhibition for one hour.

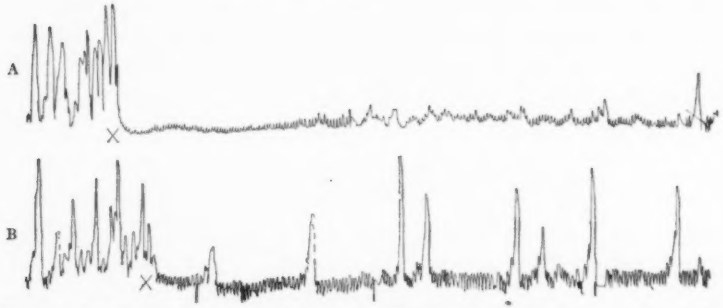

FIGURE 1. (One third the original size.) Tracings from empty stomach of dogs. *A*, normal dog; *B*, dog with both splanchnic nerves cut; *X*, introduction of 25 cc. 0.5% HCl into the stomach. Showing less complete inhibition of the hunger contractions by acid in the stomach after section of the splanchnic nerves.

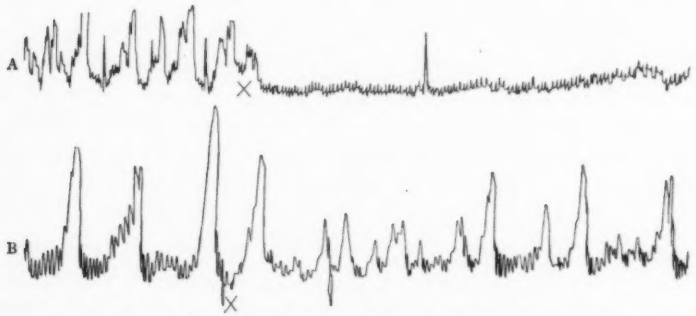

FIGURE 2. (One half the original size.) Tracings from the empty stomach of dogs. *A*, normal dog; *B*, dog with section of the vagi and the splanchnic nerves; *X*, introduction of 12 cc. of brandy + 12 cc. water into the stomach. Showing less complete inhibition by alcohol in the case of the stomach isolated from the central nervous system.

If these substances are introduced into the stomach of dogs during a period of relative quiescence and tonus relaxation the

only effect appears to be a still greater tonus relaxation and prolongation of the quiescent period. In some cases one or two hunger contractions follow immediately on introducing the material into the stomach. I am inclined to attribute these contractions to the mechanical distension of the stomach wall rather than to stimulation of nerve endings in the mucosa. This phenomenon was never observed when the stomach was in strong tonus and hunger contractions.

Typical tracings illustrating this inhibition of the gastric hunger contractions in normal dogs by acids, brandy, and beer are reproduced in Figs. 1(A) and 2(A).

III. The Action of Carbon Dioxide

The influence on the hunger contractions of CO_2 in the stomach cavity is the same in man and dog. The experiments on dogs were made with water saturated with CO_2 and with CO_2 gas. When the gas was employed, at times enough of it was passed into the stomach via the fistula to cause escape of the gas through the esophagus. The water saturated with CO_2 has practically the same action as ordinary water, that is a slight temporary inhibition without any after effect of the nature of increased tonus or contractions. This is true whether the carbonated water is introduced during active hunger contractions or during relative quiescence. The CO_2 gas usually initiates some contractions if introduced into the stomach during a period of quiescence. This is evidently due to mechanical distension of the stomach walls and not to chemical stimulation of nerve endings in the mucosa. If the empty stomach is in vigorous tonus and hunger contractions the CO_2 gas causes a slight temporary inhibition without any stimulating after effect. This temporary inhibition is in all probability due to a weak acid stimulation of the nerve endings in the mucosa.

IV. The Effects of Complete Section of the Splanchnic Nerves

The inhibition of the gastric tonus and hunger contractions by acids, alkalies, alcohol, etc., in the stomach cavity persists after section of the splanchnic nerves, but it is on the whole less complete and of shorter duration than in dogs with all the extrinsic gastric nerves intact. This applies to all the substances used in this series of experiments. A pair of tracings illustrating this diminution of the acid inhibition from the stomach after section of the splanchnic nerves are given in Fig. 1. When, as in the present series, the test with each substance is repeated at least ten times on each animal, some variations in the intensity and duration of the inhibition appear. That is to be expected, because the degree of inhibition depends on several variable factors, such as the excitability of the nerve endings in mucosa, the excitability of the Auerbach plexus and of the central nervous system, the tonus of the stomach, etc. It is therefore true that the most pronounced inhibition observed after section of the splanchnic nerves may be as marked as the feeblest inhibition obtained in the normal dogs. But when all the results in the two series of dogs are compared, there is no question but that *section of both the splanchnic nerves diminishes the inhibition following stimulation of the gastric mucosa by acids, alkalies, alcohol*, etc.

Several explanations of this fact suggest themselves. (1) Since section of the splanchnic nerves in dogs increases on the whole the tonus and the hunger contractions of the empty stomach, the diminished inhibition may be due to this greater vigor of the stomach rather than to cutting the efferent path of a long reflex. I do not think that this is the main or important factor, because the typical marked inhibition is obtained in normal dogs even when the stomach shows as vigorous tonus and hunger contractions as the maximum shown by dogs with the splanchnic nerves severed. Moreover, the inhibition is still incomplete in splanchnetomized dogs that show relatively feeble hunger contractions ("type I").

(2) The substances stimulate afferent vagi nerve endings in the mucosa, and the afferent vagi impulses via conscious or subconscious centres finally stimulate the efferent inhibitory neurones in the splanchnic system. It is well known that the vagi carry afferent fibres from the stomach mucosa and that the splanchnic nerves carry inhibitory fibres to the stomach. The present experiments give the first intimation that the afferent vagus and the efferent splanchnic systems are so intimately associated in gastric motor reflexes. It is possible that the reflex also involves the adrenal glands, so that the above inhibition is to be accounted for, in part, by the depressor action of an increased output of epinephrin.

V. The Effect of Section of the Vagi Nerves and of the Vagi and the Splanchnic Nerves

When all the records are compared it appears that section of the vagi nerves alone or section of both the splanchnic and the vagi nerves diminishes the inhibitory reflex from the gastric cavity on the whole more than does the section of the splanchnic nerves alone. A fact of greater importance, however, is the persistence of the reflex after complete isolation of the stomach from the central nervous system. *The inhibition is therefore primarily a local reflex.* The increased diminution of the inhibition after the vagi section may involve two mechanisms. It is well known that the vagi contain some efferent inhibitory fibres to the stomach motor mechanism, and may be, together with the splanchnic inhibitory fibres, involved in the long inhibitory reflex. But since the gastric tonus fibres in the vagi and the gastric inhibitory fibres in the splanchnic nerves are practically antagonistic, it is highly probable that afferent impulses leading reflexly to the stimulation of the inhibitory neurones lead at the same time to the inhibition of the tonus or motor neurones.

The reader may object that we are now discussing inferences that do not necessarily follow from the facts so far at hand. The facts, in brief, are these. The inhibition of the tonus and the contractions of the empty stomach by stimulation of the

gastric mucosa persist after isolating the stomach from the central nervous system, but the inhibition is diminished in intensity and duration after section of the splanchnic nerves, and somewhat more so after section of the vagi nerves. It has been shown that section of the vagi leaves the stomach on the whole permanently hypotonic, except during prolonged starvation, although there seems to be a gradual improvement in the efficiency of the local tonus mechanism. Is it not possible that the lessened inhibition after the vagi lesion is due to a depression of the excitability of the local afferent nerve endings in the mucosa or depression of the local reflex centre similar to the tonus depression? Our experiments do not exclude this possibility, but the results on the dogs with only the splanchnic nerves severed

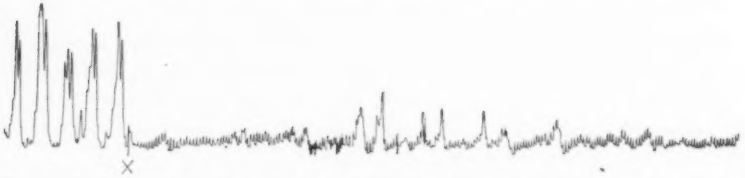

FIGURE 3. (One third the original size.) Tracing from the empty stomach of a dog six weeks after section of the vagi and the splanchnic nerves; *X*, introduction of 25 cc. port wine into the stomach. Showing a degree of alcohol inhibition almost as marked as in normal dog.

show conclusively that it is not the sole factor. For in these dogs there is no gastric hypotonus, and yet the inhibition from the gastric mucosa is diminished.

Another possibility has occurred to me. When the same quantity (25–50 cc.) of acids, alkalies, or alcoholic beverages is introduced into a stomach in tonus and into a stomach in hypotonus, it seems likely that the solutions will come in contact with more of the mucus membrane in the tonic than in the atonic stomach. This might result in less inhibition in the case of atonic stomach from the mere fact of stimulation of less of the afferent nervous mechanism. I have tested this possibility by introducing a greater quantity of the respective solutions in the hypotonic stomach. But if 25 cc. of acid or of beer fails to produce complete inhibition, 50 cc. of the same liquid usually also fails. This is to be noted, however, that the depression of

inhibition following splanchnic and vagi section is most marked for a week or two after these nerve lesions are made, and there is a distinct tendency to improvement in the efficiency of the local reflex *pari passu* with the improvement in the local tonus mechanism (Fig. 3). This is probably an instance of readjustment of local reflex mechanisms to a fair degree of efficiency in the absence of central tonus and accessory central long reflexes.

The experiments on Mr. V. and on normal dogs led to the conclusion that contractions of the empty stomach cannot be induced by stimulation of the gastric mucosa, that such stimula-

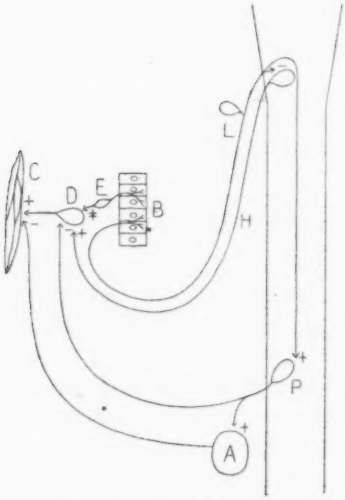

FIGURE 4. Diagram to represent the local and the long reflex mechanisms involved in the inhibition of the gastric tonus and the hunger contractions from stimulation of the gastric mucosa. *A*, adrenal gland; *B*, gastric mucosa; *C*, stomach musculature; *D*, Auerbach's plexus; *E*, local afferent neurones from the gastric mucosa to the Auerbach's plexus (these neurones are predominantly inhibitory); *H*, tonus or motor neurones to the stomach via the vagi; *L*, afferent neurones in the vagi from the gastric mucosa; *P*, neurones in the splanchnic nerves; + = stimulation; − = inhibition.

tion causes inhibition only. It was noted that one or two contractions occasionally follow immediately on the introduction of these liquids into the stomach, but it seemed probable that these contractions were due to the mechanical distension of the stomach

walls rather than to the chemical or mechanical stimulation of the nerve endings in the mucosa. These initial contractions following the introduction of acids, alkalies, or alcoholic beverages into the stomach occur more frequently in the hypotonic stomach isolated from the central nervous system. This is true even when special care is taken to introduce the substance slowly so as not to cause sudden distension of the stomach walls. I am not yet satisfied that this primary motor response is actually due to stimulation of nerve endings in the mucosa. If it is, there must be in the mucosa a few afferent nerve endings of the excitatory type, but the afferent inhibitory nerve endings are so much more numerous that the influence of the former group is completely submerged by the latter except occasionally when the stomach is hypotonic. Or else local afferent nerve endings in the mucosa are all of one type, but the type of reflex produced by this stimulation may depend in part on the tonus condition of the reflex centres (Auerbach plexus).

The local and long reflex mechanisms governing the tonus and the hunger contractions of the empty stomach demanded by the above work on dogs are diagrammatically represented in Fig. 4. It may be noted that this diagram is not intended to represent all the afferent gastric nerve components, such as those acting in various ways on consciousness, on the vaso-motor centres, etc. The adrenal glands are indicated simply as a possible factor, because conclusive data has not yet been obtained on that point.

THE TONUS AND HUNGER CONTRACTIONS OF THE EMPTY STOMACH DURING PARATHYROID TETANY

By A. J. CARLSON

[*From the Hull Physiological Laboratory of the University of Chicago*]

THE X-ray method of investigation of the gastric movements of digestion in cats and dogs in parathyroid tetany failed to reveal tetany of the stomach and the intestines.[1] The gastric and intestinal movements of digestion may continue normal during severe tetany; and in so far as the tetany condition influences the movements of digestion of the stomach and intestines, this influence is in the direction of depression (slowing and weakening). It was also found that the gastric digestion is usually retarded by the parathyroid tetany condition.

These observations have now been extended to the empty stomach by the method of an inflated balloon in the stomach introduced through a fistula of the fundus. This permits the taking of continuous and accurate records of the motor condition of the stomach in dogs.[2]

The motor condition of the empty stomach in tetany is of special interest in connection with the problem of hunger, as animals in tetany show diminished desire for food in proportion to the severity of the tetany. Hunger is caused mainly by gastric contractions. If the condition of the parathyroid tetany of the skeletal muscles involved a parallel tetany of the musculature of the digestive tract one might even expect an increased hunger in tetany. The refusal of food by animals in tetany might be accounted for if the tetany leads to gastric hypotonus and absence of hunger contractions. There is a third possibility. The normal gastric hunger contractions may be

[1] CARLSON: This journal, 1912, xxx, p. 309.

[2] CARLSON: This journal, 1913, xxxii, p. 369.

present in tetany, but the impulses from the stomach may fail to give rise to the sensation of hunger because of the change (the tetany condition) in the central nervous system.

Observations have now been completed on three dogs. The dogs were observed every third day for two weeks, so as to secure the average normal gastric tonus and hunger contractions. Complete thyroid parathyroidectomy was then made. All three dogs ran a typical course of tetany of varying severity from day to day. Dog I died in tetany on the sixth day after the operation; Dogs II and III died in depression on the eighth and tenth days respectively.

The results of the observations on the relation of parathyroid tetany to the tonus and movements of the empty stomach were practically the same in the three dogs. *In this tetany there is depression of the tonus and contractions of the empty stomach parallel with the severity of the tetany, so that during extreme tetany the stomach is practically atonic and tonus contractions and hunger contractions are completely absent.*

The milder stages of the tetany (hyperexcitability of the motor nerves, slight tremors, twitchings, and some salivation) may coexist with considerable gastric tonus and hunger contractions, but the hunger contractions are always slower and weaker than normal.

It is well known that the course of parathyroid tetany, especially in dogs, is usually more or less periodic, the animal recovering spontaneously for periods varying from a few hours to a day or more between the tetany attacks. Dog II showed two such periods of spontaneous recovery of thirty-four and twenty hours duration. During these periods the gastric hunger contractions and the gastric tonus also returned to approximately normal conditions. A typical series of tracings from Dog II showing the parallel between the severity of the tetany and the degree of the depression of the motor activities of the empty stomach are reproduced in Fig. 1.

During what might be called the very beginning of the tetany symptoms following the operation in Dog I vigorous gastric hunger contractions were present and the contractions were on the whole of a longer duration than shown by this dog before

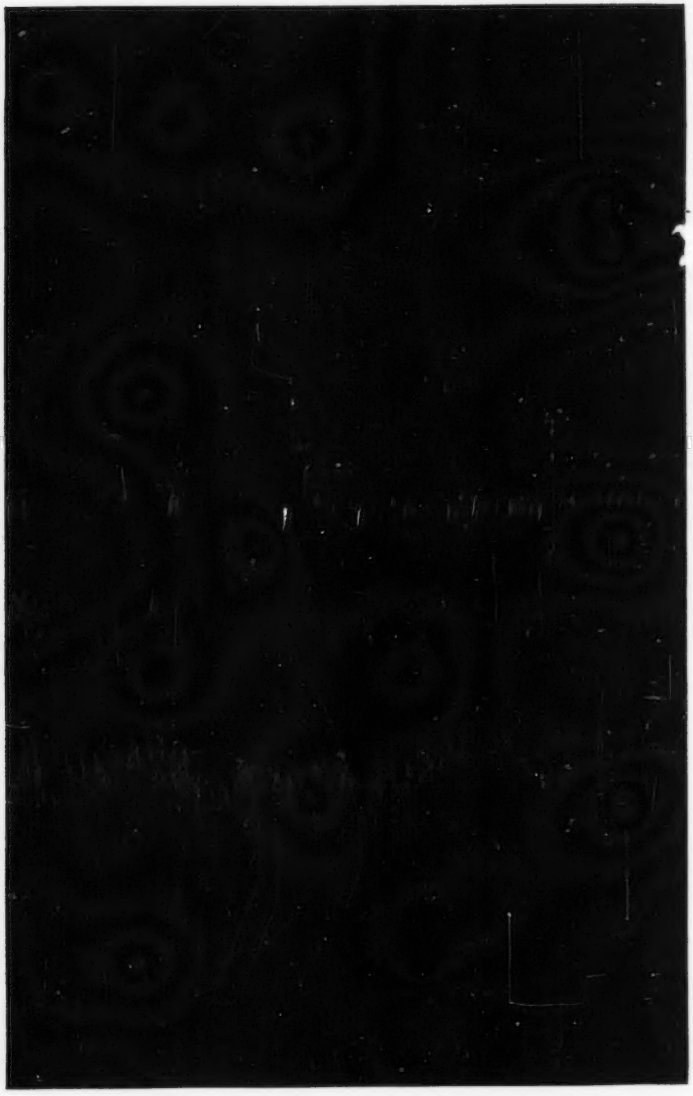

FIGURE 1

the operation. The intervals between the contractions were also relatively long. This type of contractions has been observed in normal dogs, so it is not specific for the tetany condition. Nor can the contractions be interpreted as gastric tetany. Nothing like the gastric tetany ("Type III")[3] observed, in normal animals (man and dog) especially in prolonged starvation was recorded in the tetany dogs at any stage of the tetany.

The relation of this depression of the gastric hunger contractions to the depression of the appetite is, nevertheless, not a direct one. During the mild stages of tetany the depression of the appetite is usually much greater than one would

FIGURE 2

expect on the basis of the degree of depression of the hunger contractions. In strong to extreme tetany there are no gastric hunger contractions and the dogs show no appetite, but I was surprised to find that appetite may be entirely absent even though fairly strong hunger contractions are present in mild tetany, or if the dog shows some appetite the amount of food taken is very small. Thus Dog I ate only a few grams of meat at the time his stomach showed the hunger contractions of Fig. 2. The degree of hunger contractions shown in Fig. 1B and Fig. 2 is invariably associated with relatively great eagerness for food in normal dogs. It is therefore clear that the diminution or lack of appetite in tetany cannot be accounted for solely on the basis of depression of gastric hunger contractions, although this is unquestionably one of the factors. But

[3] CARLSON: This journal, 1913, xxxii, p. 369.

we must also take into account either (1) a change in the central nervous system, or a (2) change in the character of the nervous impulses from the stomach.

On the whole it can be said that the condition of parathyroid tetany, in so far as it influences the stomach motor activities, depresses both the digestion movements of the filled and the hunger contractions of the empty stomach, but the movements of digestion show less depression than do the hunger contractions. Thus moderately strong tetany may leave the gastric digestion movements practically normal but completely inhibit the hunger contractions of the empty stomach. The tetany condition does not lead to increased motor activity or gastric tetany either in the empty or the filled stomach.

The cause of this depression of the gastric motor activities in parathyroid tetany is not determined. In the case of the digestion movements it was shown not to be due to splanchnic inhibition. This test has not been made in the case of the hunger movements. But one factor in the depression or complete inhibition of the hunger contraction in tetany *is the increased excitability of the nerve endings in the gastric mucosa.* Vomiting is a tetany symptom in dogs. And dogs in tetany frequently vomit with nothing in the stomach but bile or saliva. In such dogs water at body temperature introduced into the stomach through a fistula in the fundus causes vomiting. The presence of a delicate rubber balloon in the stomach, or the slight inflation of the balloon causes vomiting. This never occurs in normal dogs. The stimulation of the nerve endings in the gastric mucosa in normal animals (man and dog) causes inhibition of the gastric hunger contractions through local and long reflexes. In parathyroid tetany these nerve endings in the mucosa became so hypersensitive that they are intensely stimulated by saliva, water, bile, and gastric juice. But in addition to these inhibitory reflexes from the gastric mucosa we probably also have a direct depression of the automatic tissue in the stomach, for it is not likely that the inhibitory reflexes, even though very strong, could maintain the sustained extreme depression of the gastric motor mechanism seen in strong tetany.

In the normal dog sudden inflation of the balloon in the fundus leads to one or two strong contractions of the empty stomach. In dogs in marked tetany this leads to vomiting. Vomiting thus induced, as well as spontaneous vomiting in these tetany dogs, seems to cause some increase in gastric tonus lasting for a minute or more after cessation of the vomiting. This increased tonus may, of course, be only apparent, and due to an increased tonus of the abdominal muscles. But I am inclined to interpret the tracings as above, as I have evidence in man that strong contractions of the abdominal muscles, as in forced respiration, induce contractions of the empty stomach, probably by an associated innervation through the tonus fibres of the vagi.

When the parathyroid tetany is temporarily suppressed by intravenous injections of calcium salts (calcium lactate) the calcium injections are followed by marked depression of the tonus of the empty stomach and inhibition of the hunger contractions, if these are present. The stomach recovers from the calcium inhibition in five to fifteen minutes. The calcium inhibition of the motor activity of the empty stomach is probably due mainly to a direct depression of the automatic tissues in the stomach.

Summary

1. Parathyroid tetany in dogs does not lead to increased tonus or contractions of the empty stomach, but to depression of the tonus and the hunger contractions. The degree of depression of the motor activities of the empty stomach is on the whole parallel with the severity of the tetany symptoms, and more marked than the depression of the gastric movements of digestion.

2. The hyperexcitability of the nerve endings in the gastric mucosa is a factor in this depression. The stimulation of these nerve endings leads, through local and long reflexes, to inhibition of the tonus and the hunger movements. There is probably also a direct depression of the automatic tissue in the stomach through changes in the blood.

3. The diminution of or lack of appetite for food in animals in tetany is on the whole greater than would be expected on the basis of the degree of depression of the gastric hunger contractions. The cause of the lack of hunger and appetite in tetany is therefore complex. It is due in part to the depression of the gastric hunger contractions. Other factors are the change in the brain, and in the character of the afferent nervous impulses.

THE EFFECT OF PITUITARY EXTRACT UPON RENAL ACTIVITY

By C. E. KING and O. O. STOLAND

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IT is well known that extracts of infundibular portion of the hypophysis, when injected intravenously, give rise to an increased flow of urine. Schäfer and Herring [1] assert that this effect is due to a specific stimulation of the renal epithelium by a substance contained in the extract. These observers have also suggested the possibility that the diuresis might be due to an increased blood supply to the kidney resulting from vaso-dilation. They have, however, been inclined to the former view because in a number of cases they obtained diuresis without dilation of the kidney, in fact, with constriction. Houghton and Merril [2] reach the conclusion that the diuresis is due primarily to the increase in blood pressure brought on by general vaso-constriction.

The experiments reported in this paper were undertaken at the suggestion of Dr. S. A. Matthews, to determine, if possible, which of these hypotheses is correct, and to throw more light upon the mechanism involved.

All our experiments were performed on dogs. Schäfer and Herring [1] report that their results with dogs were not as constant as with other animals. We found, on the contrary, that under like conditions the constancy and uniformity of effect was very striking. There were, however, variations in the degree of diuresis on repeated injections in the same dog, which, we believe, have yielded additional facts as to the conditions under which pituitary extracts cause diuresis.

[1] E. A. Schäfer and P. T. Herring: Philosophical Transactions of the Royal Society of London, Series B, 1908, cxcix, p. 1.

[2] Houghton and Merril: Journal of the American Medical Association, Nov. 28, 1908, p. 1849.

The dogs chosen for the crucial experiments were large healthy animals weighing from ten to eighteen kilos. They were not prepared in any special way except that in a few cases they were not given food on the morning of the experiment. The animals were anesthetized with ether only, except in a few cases when small doses of atropin sulphate were given intravenously at the beginning of the experiment. The use of atropin sulphate was not made a routine practice, because with proper vigilance the anesthesia could be made uniform without the introduction of this drug which might complicate conditions.

Records were taken of the blood pressure, kidney volume, rate of flow of urine from the right ureter, and in a number of cases records of the changes in the volume of other organs. These records were obtained by the ordinary methods. The urine was always collected from the ureter of the free kidney.

In all the crucial experiments the extracts were injected into the femoral vein. Three brands of extracts were used: "Pituitrin," manufactured by Parke Davis & Co.; "Infundibulum" of Burroughs & Wellcome; and an extract made by ourselves. This extract was made from dried, pulverized posterior lobes of the ox. The dried material was first extracted with hot absolute alcohol in order to remove the depressor substance, and then with hot physiological salt solution. This latter extract gave results apparently identical with those obtained from commercial brands.

Effects of the First Injection of 1 cc. of Pituitary Extract. — After the animals had been anesthetized and prepared for recording the blood pressure and changes in kidney volume, there was frequently a transient anuria, probably a reflex inhibition from injury to the peritoneal wall, ureters, and kidney. No injections were made until the urine was again flowing at a uniform rate, except in a few cases in which the urine did not begin to flow spontaneously, and the injection was made to start it. It was frequently observed that the anuria was more prolonged when the urine in the bladder was of a relatively high specific gravity.

After the injection was begun there was always a latent period averaging about five seconds. This was followed by a

rather abrupt constriction of the kidney and a rise in the systemic blood pressure. Frequently the rise in blood pressure was very abrupt, in other cases gradual. The blood pressure gradually returned to normal, the period of increased pressure lasting on an average about ten minutes. The constriction of the kidney was followed by a gradual dilation, variable in extent, but a phenomenon constant in occurrence. During the period of kidney constriction the flow of urine was greatly decreased and in most cases ceased entirely. The flow of urine began again at about the same time that the kidney reached its normal size. The rate

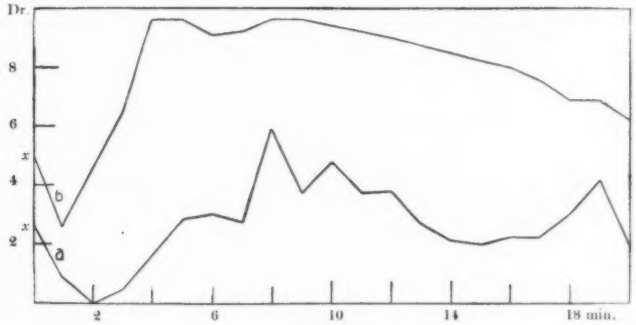

FIGURE 1

gradually increased to a rate greater than the normal, following a gradual dilation of the kidney.

This diuretic effect after the first injection of pituitary extract was obtained in every case with one exception, and in that case the right kidney was found to be atrophied and not functional. At the end of this experiment the urinary bladder, previously empty, was greatly distended, indicating that the kidney in the oncometer responded to the extract. The period of dilation and diuresis lasted on an average about twenty minutes. Following this there was a period when the rate of flow was less than normal, and the kidney became normal in size, or somewhat constricted.

Fig. 1 was constructed by averaging the results obtained on six different dogs, representing the average effect after the first injection (x) of 1 cc. of a 1 per cent extract of the posterior lobe.

(*b*) represents the relative changes in the volume of the kidney, and (*a*) the rate of flow of urine at the same time. It will be seen that the fluctuations in the rate of flow of urine were much greater than the fluctuations in kidney volume. This is accounted for by the fact that after injections there were often strong uretral contractions during which four or five drops would flow in rapid succession followed by a period of no flow until the next contraction. The systemic blood pressure nearly always returned to normal before the maximal diuretic effect was obtained. Frequently diuresis was observed with the systemic blood pressure slightly below normal. This is in agreement with the observation of others (Hoskins and Means).[1]

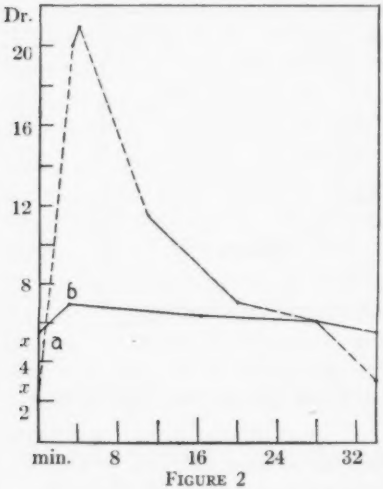

FIGURE 2

Another series of experiments was carried out, but instead of using pituitary extract, a 20 per cent solution of glucose was used. The object of this variation was to study the changes in kidney volume during diuresis produced by a substance, the effect of which upon the vaso-motor system is not marked. 25 cc. of the glucose solution caused a marked diuresis in almost every case. Fig. 2 represents the results as averaged from six dogs, (*b*) representing the relative dilation of the kidney, and (*a*) the flow of urine at the same time.

The results just described were obtained by injection of a diuretic which does not cause great vaso-motor changes. Following this it was thought advisable to test the diuretic effect of substances other than pituitary extracts which give rise to marked vaso-motor phenomena. Epinephrin was chosen in this case.

[1] R. G. Hoskins and J. W. Means: The Journal of pharmacology and experimental therapeutics, 1913, iv, p. 435.

Fig. 3 shows the effects of one-tenth of a cubic centimetre of epinephrin (1:1000); (*a*) represents the flow of urine and (*b*) the relative changes in kidney volume.

After a brief latent period there was in every case the typical rise in blood pressure, and at the same time a very marked abrupt constriction of the kidney. Both the blood pressure and the kidney volume returned rather rapidly toward the normal, the kidney becoming slightly dilated and remaining so for a period of from 10 to 15 minutes. During the period of kidney constriction the flow of urine was decreased, and in several cases ceased entirely, but with the return to normal size and slight dilation of

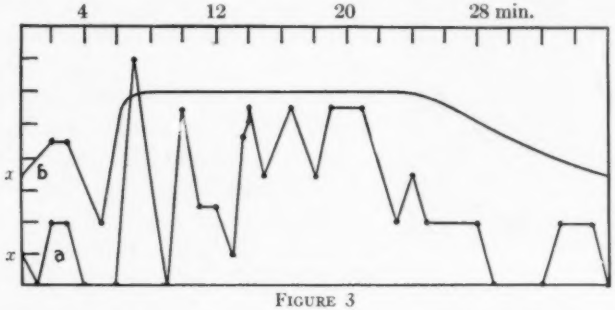

FIGURE 3

the kidney, the flow began and increased rapidly to a rate much greater than normal. This diuresis lasted until the kidney had again reached its normal size.

Finally the effects of repeated injections were studied. It has been found by others that in the dog with repeated injections there is very little or no variation in the blood pressure reaction, but that in the cat and rabbit the blood pressure is progressively less. This being the case, the dog appears to be a good animal to determine whether the diuresis is a function of the increased blood pressure. If this hypothesis were correct, then in the dog repeated injections, other conditions being constant, should give the same degree of diuresis. The graphs in Fig. 4 show the relative changes in kidney volume and urine flow in the same animal. The blood pressure curve is not represented. Our results agree with those formerly reported, — that the blood pressure response is the same each time provided the pressure is

allowed to become normal before another injection is given. It will be seen that neither the changes in kidney volume nor the diuretic effect were the same after any two successive injections. It will also be noted that the pressor effect was most marked after the first injection, and almost disappeared after the second injection. Other experiments of the same type showed the same thing. In one series the first three injections were made at brief intervals, about thirty minutes, and then an hour was allowed to pass before the next injection. After this injection the pressor effect was very marked but of short duration, and was followed

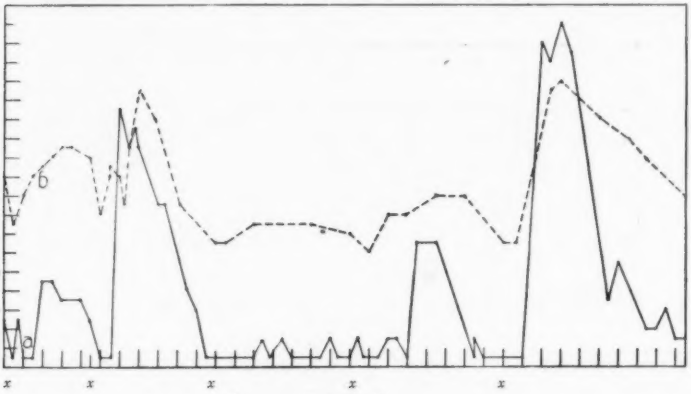

FIGURE 4

by a great dilation of the kidney and great diuresis. So great was the vaso-motor effect that typical Traube Hering waves appeared in the kidney curve. Fig. 5 is a portion of the tracing showing the contraction waves in the kidney, and also showing that the urine flowed more rapidly at intervals corresponding in time to the intervals between two successive waves.

In case great diuresis resulted, whether from the first or second injection, it was always followed by a period of urinary depression, and repeated injections failed to give rise to a rapid flow. During this period of depression either dextrose or urea caused diuresis proportional in extent to the amount injected, thus showing that the kidney's capacity for activity had not been materially lessened. We failed to obtain a single instance in

which the kidney did not dilate to some extent under the treatment just described. If on the other hand, during this period of urinary depression, both glucose and pituitrin, or urea and pituitary extract were injected the results were not so uniform. It is difficult to say for a particular injection that if pituitary extract alone or glucose alone had been injected the diuretic effect would have been greater or less than that obtained. In a few cases the diuretic effect was more marked than with dextrose alone on an average, but there was always a greater dilation of the kidney. On the other hand, in a few cases where the pituitary extract was injected after the diuresis from dextrose had begun and reached what appeared to be the maximal rate, judging from the amount of kidney dilation, the injection of pituitary extract was followed by no greater diuresis.

FIGURE 5. (One half the original size.) *A*, blood-pressure; *B*, kidney oncograph; *C*, urine flow; *D*, time in 6 seconds.

One experiment was carried out in order to determine whether the amount of urine secreted in 24 hours is greater when pituitary extracts are injected than when not. The extract was injected subcutaneously in 2 cc. doses twice daily. The volume secreted was not increased. The nitrogen content of the urine, however, was greatly increased.

DISCUSSION

A survey of the experimental data and observations here presented reveals the following important facts: (a) extracts of the pituitary gland, when administered intravenously gave rise to marked vaso-motor phenomena and diuresis; diuresis from pituitary extract occurred only in connection with dilation of the kidney; diuresis following the injection of urea and dextrose was

accompanied by dilation of the kidney, but not so extensive as in the case of pituitary extracts; other substances giving marked vaso-motor phenomena, one phase of which is vaso-dilation, gave rise to diuresis; diuresis was not directly proportional to the amount of extract injected; great diuresis was always followed by a period of urinary depression, during which time pituitary extracts acted very weakly as diuretics; during this period of urinary depression other diuretics were active; if an hour or more was allowed to intervene between injections, successive injections of pituitary extract gave rise to marked diuresis.

Our primary interest in these observations consists not so much in the mere description of them, as in their significance in connection with the mechanism involved in causing the diuresis.

The diuretic effect of pituitary extracts was observed and described by Magnus and Schäfer in 1901.[1] A little later Schäfer and Herring[2] put forward the view that the diuresis is due to a specific stimulation of the renal epithelium. This view has been accepted by a number of observers as the most plausible explanation. (Cushing,[3] Hoskins and Means).[4] Houghton and Merril[5] did not come to this conclusion, but contended that the diuresis is dependent primarily on increased blood pressure. These observers came to this conclusion after doing perfusion experiments on the isolated kidney. But perfusion of any isolated organ is less satisfactory than when intact in the animal.

Lately Gesell has shown that the pulse pressure is an important factor in renal secretion. Hoskins and Means[4] have shown, however, that pulse pressure is not an important factor in causing pituitary diuresis.

Unless we accept the "mechanical" theory of Ludwig, we are forced to assume that diuresis always results from a stimulation of the renal epithelium. In the study of each diuretic, then, the question arises whether the substance in question stimulates the epithelium directly, or whether its primary action

[1] MAGNUS and SCHÄFER: Journal of physiology, 1901, xxvii, p. 9.
[2] *Loc. cit.* [4] *Loc. cit.*
[3] HARVEY CUSHING: The pituitary body and its disorders, 1912, p. 8.
[5] *Loc. cit.*

consists in producing other effects which in turn stimulate the renal epithelium.

If pituitary diuresis is the result of direct stimulation then we should expect the same degree of successive diuresis after injection of the same amount. In our experiments, however, we found that periods of marked diuresis are followed by periods during which the same dose will not arouse the kidneys to activity. In a number of cases larger doses were administered. Only a slight diuresis resulted, but the dilation of the kidney was greater than when the original dose was administered. This failure to produce a diuretic effect cannot be attributed to fatigue of the renal epithelium, for it is known that the kidney is capable of great activity for much longer periods than in this case, and furthermore, injections of dextrose or urea never failed to produce marked diuresis.

Diuresis should also occur even if there is no renal dilation. We were not able to obtain such a result. In all our experiments constriction of the kidney was accompanied by a slowing of the urinary flow, and in cases of great constriction, by anuria.

The contentions of Houghton and Merril,[1] in the light of our results do not account fully for the effects obtained. If pituitary diuresis is a function of the blood pressure, the kidney should be the most active at the time of highest blood pressure. The greatest flow of urine usually came after the blood pressure had almost returned to normal, and in a number of cases great diuresis occurred with the systemic blood pressure slightly below normal. It cannot be denied, however, that blood pressure may be an important factor in bringing about diuresis or anuria. It was repeatedly observed that when the systemic blood pressure rose considerably the kidney increased in size and the flow of urine was accelerated. On the other hand, it was repeatedly observed that when the blood pressure fell considerably, even in the midst of pituitary diuresis, the kidney became smaller and the flow of urine diminished.

It should then be asked why increase in blood pressure does not cause diuresis. This is a very vital point, because we observed that with repeated injections of pituitary extract, the

[1] *Loc. cit.*

blood pressure reaction is approximately the same after each injection, but that the diuretic effect is very variable. It is significant in these cases that the reaction of the kidney as to its size is also variable, and that the relative degree of diuresis and kidney dilation run parallel.

The kidney is an organ which normally is active to such an extent that the composition of the serum as to waste products remains fairly constant. Urea and water may be considered as normal diuretics, and as the substances increase or decrease in the blood, the kidneys are stimulated to more or less activity. If the blood is low in metabolites and waste substances it takes a stronger and more persistent stimulus to force the kidney to lower the composition still more. This is suggested by our repeated observations that in case the flow of urine was slow at the start, and if the urine in the bladder was of high specific gravity, the first injection of pituitary extract gave comparatively little diuretic effect. It also partially explains the fact that after strong diuresis there was a period of more or less anuria.

It is well known that the kidney can be made more active by an increase in its blood supply. An increase in the general blood pressure will increase the blood supply to the kidney, provided there is no local constriction. This might be suggested as one of the initial factors in bringing about diuresis in case of pituitary extract, were it not for the marked vaso-constriction during the period of highest blood pressure. The blood supply to the kidney during the period is diminished as is also evidenced by the anuria. But others have observed an increase in the force of the heart beat after the initial vaso-constriction. This was confirmed in our observations, and in addition we frequently noted an increase in rate. These two factors will keep the blood pressure up. The vaso-constriction is of comparatively short duration. Then, following this, with a high blood pressure, the blood supply to the kidney is increased. It is not improbable that this increase of blood pressure was one of the potent stimuli in bringing about diuresis after pituitary extract, and that it failed to excite the kidney when the substances to be eliminated were present in the blood in low concentration. This is suggested by the fact that the kidney was most refractory after prolonged diuresis.

This view is also supported by the fact that epinephrin produces vaso-constriction and increases blood pressure even more than pituitary extract, but that the diuresis following its administration is less on an average.

None of the facts and hypotheses stated above account for the dilation of the kidney so characteristic after pituitary extract. We have recorded our observation that with other diuretics such as dextrose and urea there was always some renal dilation, but this dilation was not comparable in extent to that after pituitary extract. The facts at hand indicate activity on the part of the vaso-dilator nerves of the kidney, and the existence, origin and some of the reactions of these nerves have been demonstrated by Bradford.[1] Whether the pituitary extract acts upon both constrictors and dilators, the former fatiguing more rapidly, or whether it acts first upon the constrictors and later upon the dilators we do not know. We hope to throw more light upon the point by further researches. It is not improbable that there is in the kidney a local mechanism whereby increased activity caused by any stimulus can call forth vaso-dilation and consequently increase the blood supply to the organ.

It was observed that usually the blood pressure had fallen to normal or below by the time the kidney had reached its maximum dilation. This fact in itself suggests that the fall in pressure was at least in part due to the increased blood supply to the kidney. Ranke[2] has estimated that under normal conditions 1.63 per cent of the blood is in the kidney at a given time, say one minute. Landergren and Tigerstedt[3] have shown that in strong diuresis as much as 5.6 per cent of the blood may pass through the kidneys in one minute. The difference is enough to account for the fall. The fact that the fall in pressure is greater and more abrupt in case the kidney dilation is greater and more abrupt also supports this view.

Thus it appears that pituitary diuresis may be due to a num-

[1] J. R. Bradford: Journal of physiology, 1889, x, p. 358.

[2] Quoted by Howell. Taken from Vierordt's "Anatomische, physiologische und physikalische Daten und Tabellen," Jena, 1893.

[3] Landergren and Tigerstedt: Skandinavisches Archiv für Physiologie, 1892, iv, p. 241.

ber of co-existent factors. There is following its administration an increased blood pressure and a vaso-dilation of the kidney. These two factors, either combined or separately, will increase the blood supply to the kidney, which condition is admitted to be a stimulus sufficient to increase the activity of that organ, provided the substances in the blood to be eliminated are not too low in concentration. To these should be added the presence of a local reflex mechanism whereby increased activity brings about vaso-dilation, thus completing the ideal condition for greater renal activity.

While we do not maintain to have shown how the pituitary extract acts upon the kidney, the following evidence inclines us against the view that it acts by direct stimulation of the renal epithelium.

(*a*) We were unable to obtain diuresis without vaso-dilation in the kidney.

(*b*) There were periods during which the kidney responded to urea and dextrose but not to pituitary extracts.

(*c*) The vascular changes after administration of pituitary extract are sufficient to account for the diuresis.

We wish to express our thanks to Dr. S. A. Matthews for encouragement and valuable suggestions and criticism throughout this work.